EVALUATION OF SOME IMPORTANT ASPECTS OF SEED VIGOUR AND VIABILITY IN SOYBEAN

(*Glycine max* L. Merrill)

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DEDICATED TO:

My parents,

My wife Rehana Yasmin,

Our son Usman.

DECLARATION

I hereby declare that this thesis has been composed exclusively by me. All the research work was performed and described by me, except where acknowledged.

EHSANULLAH

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ABSTRACT

This study is in three sections. The first section investigated the environmental and seed quality factors affecting seed vigour and viability in soybean. In addition an experiment was performed which attempted to correlate laboratory viability tests to field emergence. The second section studied the effect of ageing and of the rate of water uptake on germinability using polyethylene glycol (PEG). Finally in section three numerous soybean cultivars were evaluated for storage potential and resistance to imbibition damage.

Laboratory germination at 25°C, 30°C or 35°C, seedling fresh weight, leachate conductivity and tetrazolium chloride topographic staining tests were highly correlated with field emergence at North West Frontier Province (NWFP), Agricultural University Research Station, Mingora, Pakistan. However, seeds aged for more than 4 days progressively lost the ability to germinate under field conditions compared to laboratory germination. Sealed storage at 8% seed moisture content was superior to storage at 12 or 16% seed moisture content. Compared to seeds with 12 or 16% initial moisture content those at 6% initial moisture showed reduced germinability. Reduced germinability of seeds at 6% moisture content was associated with high solute leakage. Solute leakage increased as soaking temperature increased, but at a higher rate when initial seed moisture content was low (6%). Smaller seeded cultivars were susceptible to etching (cut on both the cotyledons), but showed better storage potential compared to large seeded cultivars.

Accelerated ageing (41°C; 95-100% RH for 3 days), and soaking for 5 or 10 h in distilled water produced fewer normal seedlings and delayed 50% emergence by 1.5 days probably due to high solute leakage. The seeds germinated earlier and produced more normal seedlings when soaking occurred in 25% PEG compared to distilled water. Seeds with lower vigour due to ageing, produced a higher number of normal seedlings, greater shoot length, shoot fresh weight and shoot dry weight when pre-imbibed in 25% PEG compared to distilled water. Osmotic stress applied by means of 15, 20 or 25% PEG drastically reduced germination and seeding fresh weight. Stress applied with 10% PEG did not reduce germination, but seedling fresh weight was lowered by about 20% compared to the control. Reduced germination and shoot fresh weight were associated with osmotic stress.

Cultivars that were resistant to accelerated ageing were also resistant to soaking injury and *vice versa*. Generally, smaller or yellow seeded cultivars performed better with between 16 and 28% more normal seedlings being produced and 13 to 19% lower leachate conductivity after accelerated ageing. However, larger or light coloured seeds had poor germinability after accelerated ageing or soaking in distilled water. Solute leakage was higher from the larger seeded cultivars compared to the smaller and medium seeded cultivars. Medium seeded cultivars generally leaked more solutes than smaller seeded cultivars, but this relationship was not entirely consistent. The superiority of smaller seeded cultivars over larger seeded cultivars after accelerated ageing and against soaking injury was mostly associated with hard and waxy type seed coats.

CHAPTER 1

INTRODUCTION

1.1. SOYBEAN IN PAKISTAN

Pakistan is deficient in edible oil and protein. Domestic edible oil production meets only 19% of the total edible oil requirement in the country. To fill the gap between domestic production and total consumption the government has to import a large quantity of edible oil spending huge amount of foreign exchange (Agricultural Statistics of Pakistan, 1991).

The principal indigenous oil seed crops that contribute to the production of edible oil include, cotton (*Gossypium herbaceum*) followed by rape (*Brassica napus*) then mustard (*Sinapis alba*) and finally peanuts (*Arachis hypogea*). Most of the domestic production of peanuts is consumed as roasted nuts or used in confectionery (Akhtar, 1985). Soybean (*Glycine max* L. Merrill) and sunflower (*Helianthus annuus*) were added to the list of oil seed crops for commercial cultivation in the late 1970's. However, due to its satisfactory oil (20%) and high protein content (42%) soybean was preferred to sunflower (PARC, 1990).

Soybean has shown a high production potential on research fields both as a spring and summer crop. The crop is reported to be suitable for about 1.5 million hectares of land left barren after harvesting cotton and rice in the provinces of Punjab and Sind (Figure 1.1). Moreover, soybean is reported to be the most desirable replacement for maize and some other undesirable crops grown in the orchards. Being leguminous it can add to the fertility status of the soil, if inoculated with appropriate culture of *Rhizobium japonicum* (Khan & Qayyum, 1982).

Figure 1.1. Map of Pakistan showing potential soybean growing areas.
(Khan & Qayyum, 1982)

Soy-protein in common with other legumes is low in methionine and cysteine. However, due to its high tryptophane content it is a valuable food for normal growth of children and mammals (Hinson & Hartwig, 1982).

Since being recommended for commercial cultivation in late 1970's the area under soybean is fluctuating between 1000 to 3000 hectares (Agricultural Statistics of Pakistan, 1991). Conversely, the area under soybean in India increased from a few hundred hectares in 1971 to more than 0.5 million hectares in 1980 (Tedia, 1982). Since 1980 soybean cultivation has covered about 2 million hectares in India (FAO Yearbook, 1991). One of the major reasons for this sad state of affairs is that farmers in the plains of Pakistan are facing a serious seed viability problem.

In Pakistan some research work on soybean started during 1939 in the province of Punjab and in 1960 in the province of Sind. However, formal research was initiated in Peshawar, North West Frontier Province (NWFP) in 1965. The purpose of this research was to improve local types and introduce better exotic lines. As a result two exotic lines Bragg and Lee obtained from USA were found very promising. The results on research fields were so encouraging that the government in the mid seventies imported 5 tonnes of soybean seed from the United States of America for testing in farmer's fields. Due to inadequate knowledge of the farmers regarding the storage and cultivation of soybean the imported seed produced unsatisfactory crop stands. This acted as a disincentive for farmers and the government alike (Akhtar, 1985).

In fact soybean seeds from a spring crop are subject to field weathering at maturity in June. Moreover, during the months of July, August and September the seeds experience unfavourable storage conditions (high relative humidity and high temperature) that encourages seed deterioration, especially if seeds are stored in moisture pervious containers. Kharif (summer) planting in June is often discouraged by high soil temperatures (between 35°C to 40°C) and soil moisture stress that can

results in poor crop stands. The unpredictability of the weather in Pakistan during June, July and August, often leads to torrential rains soon after planting that may cause severe imbibition injury to the seeds and also encourages soil crust formation. Obtaining a consistently good plant stand is therefore particularly difficult in the plains of Pakistan.

To increase the production of soybean in Pakistan, a source of high quality seeds must be established, maintained and made available to the farmers with proper instructions and guidance on successful and profitable production technology. However, viable seed production in the plains of Pakistan needs sufficient scientific research to provide appropriate technological answers to the problem.

1.2. NUTRITIVE VALUE AND GENERAL IMPORTANCE OF SOYBEAN SEEDS

The soybean seed is composed of proteins, lipids, carbohydrates and minerals. However, protein and lipids are the two most important constituents of its seed. Seed composition varies with genotype, the soil and climatic conditions (Purseglove, 1968).

Variations caused by environmental effects and varietal characteristics result in protein levels ranging between 30 to 46%, and lipid levels ranging between 12 to 24% (Orthoefer, 1978). However, most of the improved varieties contain between 40 to 42% protein and 17 to 20% lipid on a dry weight basis (Hinson & Hartwig, 1982). Soybean oil is made up of saturated (palmitic, stearic) and unsaturated fatty acid (linoleic acid ranging between 45 to 55%, oleic acid ranging between 30 to 35%, and linolenic acid ranging between 5 to 10%).

Soybean is an excellent source of protein and oil, in contrast to cereals that contain no oil and only 20% protein (Hardy & Havelka, 1975). Although in 1984 the total world production of soybeans was about 17% by weight compared to that of wheat (*Triticum aestivum*), the total production of protein and lysine from soybean was

more than one-half and more than double, respectively, compared to that from wheat (Brady, 1988).

Soybean oil accounts for 20 to 25% of total world fat and oil production and 30 to 35% of total edible vegetable oil production (Smith & Huyser, 1987). Soybean oil is used for salad dressings, cooking, in margarine, whipped toppings, coffee whiteners, icings, ice cream, food shortenings, frozen desserts, soups and for industrial purposes including the manufacture of soap, paints and varnishes (Hume *et al.*, 1985). Soybean seeds are also consumed as a vegetable; dried seeds are eaten whole, split or sprouted.

Soybean meal is a large component in livestock and poultry feeds world-wide. Modern feeding practices have boosted world protein feed requirements. As a result the relative importance of soybean meal in the international marketplace has also increased dramatically (Smith & Huyser, 1987).

Today soybean seeds are the cheapest source of good quality edible vegetable protein (Campbell, 1979). Dependence on soybean for food and feed has increased rapidly in potential soybean growing tropical and subtropical countries. Nutritionists believe that the utilisation of soybean should continue to provide better nourishment for people throughout the world (Judd, 1970).

Cultivated soybean (*Glycine max* L. Merrill) attracted world-wide attention after numerous introductions into Europe and the USA in the 1920's, and thereafter (Probst & Judd, 1973). The cropped area in USA and Brazil expanded very rapidly once soybean became established as an oil rich crop with protein rich residues. Between late 1960's and early 1980's, for example, world production more than doubled mainly because of increase in output from the USA and Brazil (Hume *et al.*, 1985). Four major producers (USA, Brazil, China and Argentina) together account for 90 to 95% of world soybean seed production (Smith & Huyser, 1987).

Symbiotic nitrogen (N_2) fixation (the enzymatic reduction of N_2 gas to NH_4^+) in

legume root systems is a unique process. Grain legume crops including soybean can produce about 250 Kg of fixed nitrogen per hectare, annually in the presence of effective strains of specific rhizobia (Roughley, 1980). Thus high yield and high protein content in soybean can be achieved with a minimum of inorganic nitrogen. Subsequent crops grown in rotation with soybean may also benefit from the residual nitrogen fixed by soybeans.

Increasing the availability of symbiotically fixed nitrogen is of great importance because fertiliser nitrogen is scarce in potential soybean growing tropical and subtropical countries and is also a major expenditure for the farmers. In the USA alone, for example, the approximate value of fertiliser nitrogen in 1974 was 800 million dollars (Hardy & Havelka, 1975).

1.3. TAXONOMY OF SOYBEAN

Soybean (*Glycine max* L. Merrill) is a member of the family Leguminosae, sub-family Papilionoideae, and genus *Glycine* (Hinson & Hartwig, 1982). The name *Glycine* is derived from glykys, a Greek word meaning sweet. The cultivated form of soybean has been known by several names such as *Phaseolus max* L., *Dolichos soja, Soja hispida*, *Soja japonica*, *Glycine soja*, *Soja angustifolia*, *Glycine ussuriensis*, *Soja max*, and *Glycine max* L. Merrill (Hymowitz & Newell, 1981).

Ricker & Morse (1948) presented evidence that the only acceptable technical name of soybean under the rules of the International Botanical Congress should be *Glycine max* L. Merrill. Their conclusion was generally accepted. The name *Glycine soja* is often confusing for two reasons: (a) *Glycine max* was formerly known by *Glycine soja*, and (b) in the literature *Glycine soja* has incorrectly been called as *Glycine ussuriensis* (Hinson & Hartwig, 1982).

Recognition of *Glycine soja* as the appropriate designation for the wild soybean

dates from 1979 (Verdcourt, 1979). *Glycine max* has not been found growing wild. It probably originated from *Glycine soja*. *Glycine soja* grows wild in the Yangtze River Valley, the northern and northeastern provinces of China, adjacent areas of the USSR, and in Korea and Japan (Hinson & Hartwig, 1982). Hinson & Hartwig (1982) further reported that *Glycine max* and *Glycine soja* have diploid chromosome number, i.e. 40. Moreover, crosses between *Glycine max* and *Glycine soja* can be easily made and F_1 hybrids are fertile. This provides enough evidence to support the idea that *Glycine soja* is the wild ancestor of the cultivated form *Glycine max* L. Merrill.

1.4. ORIGIN OF SOYBEAN

The origin of the cultivated form of soybean (*Glycine max* L. Merrill) is not precisely known. "The soybean is native to southeast Asia" is a statement frequently transferred from one publication to another (Herbert & Bernard, 1963). In a region extending from northern India, Nepal and Bhutan through northern Pakistan into Afghanistan, small dark seeded primitive types were grown and might be areas of ancient cultivation (Akhtar, 1985).

A theory based on isoenzyme experimentation suggested Australia as a probable centre of dispersal for the whole pacific region including China, with migratory birds acting as seed carriers (Broue *et al.*, 1977). However, Russian breeders think that the cultivated form of soybean was the result of centuries of breeding and selection in an ancestral form similar to *Glycine soja*. Australian species were not involved in this process (Ala *et al.*, 1976).

Soybean has a long history as a crop for human food and animal feed in the orient, but elsewhere there has been significant production only recently (Hymowitz & Newell, 1981). Nagata (1959), Hymowitz (1970), and Norman (1978) agree that the most convincing and generally accepted place for the origin of soybean is in the north

and central regions of China.

1.5. DISTRIBUTION OF SOYBEAN

Soybean expanded throughout China and peninsular Korea by the first century AD. Its expansion into Japan, southeast Asia and south-central Asia occurred between the first to the 16th century AD (Hymowitz & Newell, 1981).

The first published evidence of soybean in the western hemisphere appeared in the writings of the German botanist, Engelbert Kaempfer in 1712 (Bening, 1951). Seeds of soybean sent from China by missionaries were planted at the Royal Botanic Garden Kew, London, in 1790 (Morse, 1950).

Soybean was introduced into the United States of America in 1804 (Hammond *et al.*, 1951). In the early stages of its introduction into the United States, soybean was mostly grown as a forage crop. However, the Americans soon realised its food value and started harvesting it for seed. Today the United States of America is the biggest producer and exporter of soybean seed in the world (FAO Yearbook, 1991).

1.6. PLANT CHARACTERISTICS OF SOYBEAN

All leaves except for the unifoliate first pair, are trifoliate, but occasionally leaves may have 4 to 5 leaflets (Hinson & Hartwig, 1982). Flowers are borne on short racemes originating in the leaf axils (Figure 1.2). Each raceme bears 3 to 15 small, purple, pink or white flowers. Self pollination with only 0.5 to 1%, out-crossing is the rule (Weiss, 1983).

Soybean plants develop a tap root system penetrating to a maximum depth of 1.5 to 2 m. Knowls (1973) reported that in soybean root development ceases as seed development begins. Moreover, most of soybean varieties are pubescent.

Soybean seeds are borne in pods. Each pod contains 1 to 4 seeds. Seed shape

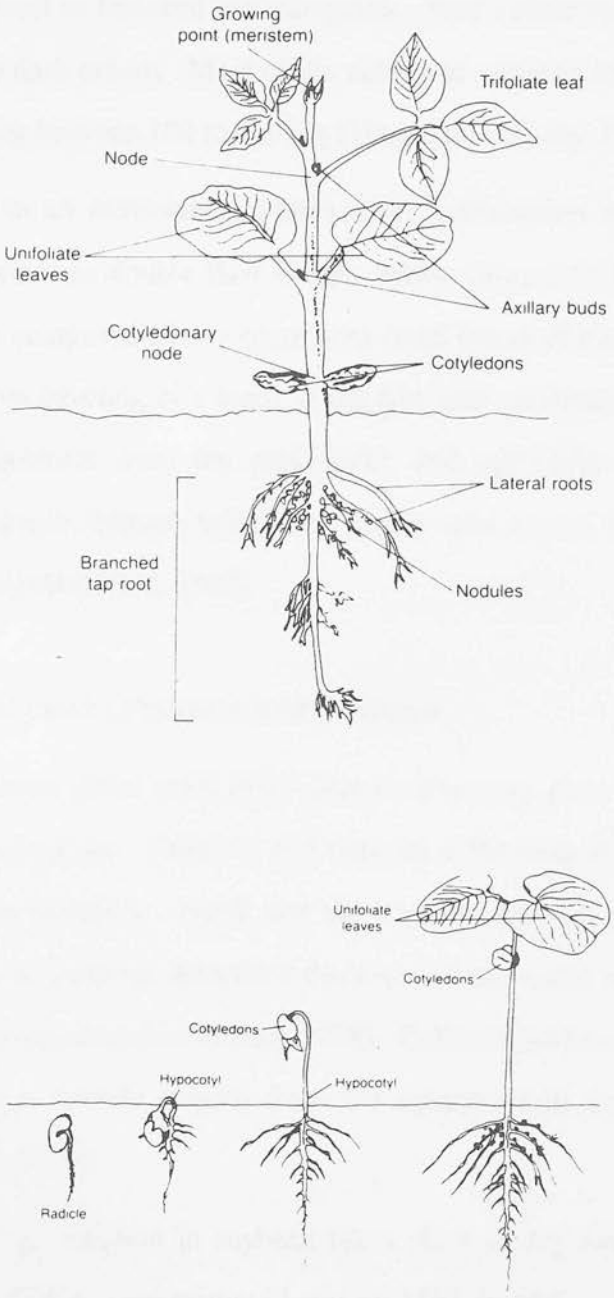


Figure 1.2. (Top) Vegetative structures of a young soybean plant, and (bottom) stages of soybean germination, emergence and seedling establishment.

Helsel (1982)

varies from spherical to flattened and elongated. Seed colour varies from yellow to black or green to dark brown. Most of the cultivated varieties have yellowish seeds, each seed weighing between 100 to 200 mg (Hinson & Hartwig, 1982).

When placed in an environment optimum for germination most soybean seeds imbibe enough water to double their weight within about 3 h (Hinson & Hartwig, 1982). Seeds are composed of two cotyledons (seed leaves of the embryo), seed coat or testa (protective covering of a seed), hilum (the scar remaining on the seed at the place of its detachment from the seed stalk), and micropyle (the integumentary opening of the ovule through which the pollen tube enters before fertilisation). Germination is epigeal (Helsel, 1982).

1.7. AGRONOMIC CHARACTERISTICS OF SOYBEAN

Environmental factors affect grain yield, days to flowering, plant height, oil content, seed size and seed colour. Seasonal and regional differences in photoperiod, night temperature, solar radiation, rainfall and the way the soybean plants interact with these environmental variables determine the areas of the world suitable for soybean production (Shanmugasundaram & Kuo, 1978). Cultivars have been developed which are adapted over a latitude ranging from the equator to as far north as Sweden (Tanner & Hume, 1978).

Maximum flower initiation in soybean takes place at day temperatures between 23°C to 29°C and night temperatures between 18°C to 28°C. Temperatures at or lower than 12°C, inhibit flowering. Temperature above 40°C, reduces growth rate, flower induction and pod retention (Hinson & Hartwig, 1982). Longer photoperiods after flower initiation may reduce or even prevent the opening of flowers (Parker & Borthwick, 1939).

The majority of modern varieties of soybean are short day annuals. Some

cultivars, however, are quantitative long day plants, yet other cultivars are day neutral. Generally, the late maturing varieties are short day plants (Hinson & Hartwig, 1982). In the field, soybean starts flowering when the days are shortened below a critical value (Carter & Hartwig, 1963; Howell, 1963).

When soybean is planted at a latitude or during a season when the photoperiod is shorter than its critical photoperiod, it will flower and mature early (Hinson & Hartwig, 1982). Biloxi, a maturity group VIII soybean genotype can be stimulated to initiate flower primordia as early as two weeks after planting, an age that coincides closely with the appearance of unifoliate leaves (Borthwick & Parker, 1938).

The intensity of light, light quality and the interaction of light and temperature is important in determining soybean growth, pest attack and subsequent seed quality (Whigham & Minor, 1978).

There are two types of stem growth habits and flower initiation patterns in soybean. Determinate varieties complete their vegetative growth before flowering. Indeterminate varieties continue to increase in height for several weeks after flowering (Hinson & Hartwig, 1982). This means that describing a soybean variety as early, medium or late maturing will possibly be unreliable, if not related to a specific location and uniform planting date.

The crop is sensitive to rainfall and humidity during flowering and fruiting, but fairly resistant to both these agents in the mature stage (Hinson & Hartwig, 1982). Because, soybeans originated in the temperate parts of China, some observers may assume that it has a peculiar adaptation to temperate climates. However, the crop can be grown equally well in tropical and subtropical zones (Pasaribu & Mclntosh, 1983).

CHAPTER 2

LITERATURE REVIEW

"Seeds are uniquely equipped to survive as viable regenerative organisms until the time and place are right for the beginning of a new generation; however, like any other form of life, they cannot retain their viability indefinitely and eventually deteriorate and die. Fortunately, neither nature nor agricultural practices ordinarily requires seeds to survive longer than the next growing season, though seeds of most species are able to survive much longer under proper conditions" (Copeland & McDonald, 1985b).

2.1. SEED QUALITY

The concept of seed quality is comprised of several important and different aspects. Within the seed industry, for example seed quality is related to suitability for malting or digestibility, etc. However, a quality seed to the farmer is one that will produce a healthy seedling possessing the ability to form an established plant, and ultimately result in economic yield (Carver, 1980).

Seed quality is maximum at physiological maturity (Singh & Gupta, 1982; Ellis *et al.*, 1986). The time between physiological and harvest maturity represents a period of seed storage in the field. During this period, seed quality is gradually lost at a rate that is mainly dependent upon infection by fungi, seed moisture content or relative humidity, and temperature. Gove (1965) named this falling from high seed quality to low seed quality as seed deterioration. Abdul-Baki & Anderson (1972) concluded that seed deterioration causes an irreversible degenerative change in the quality of seed after it attains its maximum quality level.

The highest quality level for a seed is the theoretical maximum attained under most favourable interactions between the genetic make-up of a seed and the environment

under which it is produced (Abdul-Baki & Anderson, 1972). However, the attainment of the theoretical maximum during seed development and maturation can be constrained by many environmental factors.

Individual seeds possess certain quality characteristics that include traits such as genetic and chemical composition, viability, vigour, physical condition, seed size, seed coat, seed colour and seed health. However, when seeds are combined into a seed lot, contamination by weed seeds, inert material, seeds of other crops or other cultivars of the same crop may occur. For example according to the UK Ministry of Agriculture a quality cereal seed should: (1) be true to type; (2) possess a good germination capacity; (3) not contain seeds of other cereal species; (4) be free from weed seeds and other extraneous matter; (5) be graded to a uniform size; (6) be free from seed-borne diseases; and (7) be chemically treated (Carver, 1980).

The above seven quality characteristics are important, however, the most recognised and accepted index of seed quality is the ability to germinate (Tekrony *et al.*, 1987). Germination is a physiological process that begins with water uptake (imbibition) and has been defined by Berlyn (1972) as the sequential series of morghogenetic events that result in the transformation of an embryo into a seedling.

Germinability is measured using standard tests. The procedures followed when conducting a germination test have been carefully evaluated and standardised by the International Seed Testing Association (ISTA, 1985). The commercial criterion of germination is the production of normal seedlings. Normal seedlings fall into the following categories: (a) intact seedlings; (b) seedlings with minor defects; and (c) seedlings with secondary infections.

ISTA rules (1985) do not include details of individual crop seedlings. However, it has been reported that in soybean the loss of unifoliate leaves is less damaging to yield than the loss of cotyledons. For example, Helsel (1982) reported that if both

unifoliates are lost, cotyledons compensate for 60% of this loss, but loss of both cotyledons and unifoliates decreases yield by 7%. Moreover, damaged roots also reduce yield. However, it is important that a normal seedling should show potential for continued and rapid development into a satisfactory plant under favourable environmental conditions.

Copeland & McDonald (1985b) believes that like any other form of life, seeds cannot retain their high initial quality indefinitely and eventually have to deteriorate (fall from higher quality to lower quality level) and die. However, the rate of falling from a higher to a lower quality depends upon initial seed quality at harvesting, mechanical damage during harvesting or postharvest handling and environmental conditions during storage.

The loss of capacity to germinate is almost the last event that happens as a seed deteriorates (Roberts, 1972). Therefore the standard germination test does not take into account the very substantial loss in performance potential that occurs before germination capacity is lost and it over-predicts field emergence by varying percentages depending on the relative adversity of soil conditions (Tekrony & Egli, 1977).

2.2. THE CONSEQUENCES OF SEED DETERIORATION

Seed deterioration is a major problem in agricultural production, particularly in those geographic regions of the tropical and subtropical countries where high temperature and high relative humidity prevail during periods of seed maturation and storage. McDonald & Nelson (1984) recognised that an enormous piece of research work has been performed to study the role of deleterious alterations in cellular physiology or biochemistry, influenced by environmental factors such as temperature, relative humidity and ambient oxygen, and seed characteristics such as seed size, seed colour, and seed coat structure that determine the rate of these alterations and overall quality

of seeds.

Delouche *et al.* (1973) defined seed deterioration as an irreversible process that varies among seeds in a population. However, Cherry & Skadsen (1984) concluded that seed deterioration is a continuous biochemical process and affects the pre-existing systems in a chemical way. Potts *et al.* (1978) stated that extensive chemical degradation lead to biochemical incompetence and hindrance under germination conditions, whereas germination and growth require a well balanced and precisely timed effort of all the biochemical systems in the seed.

Powell (1988) in her review on seed vigour and field establishment concluded that seed deterioration is the major cause of reduced vigour and viability in many crop species. Moreover, seed ageing involves the process of deterioration (accumulation of irreversible degenerative changes until the ability to germinate is lost).

A germination test is a standard and reliable criteria to monitor the extent of deterioration that has occurred in a seed lot. Toole *et al.* (1948) reported about half a century ago that some of the important manifestations of seed deterioration are changes in seed colour, delay in germination, decreased tolerance to sub-optimal environmental conditions during germination, lowered tolerance to adverse storage conditions, higher sensitivity to radiation treatments, reduced growth of seedlings, reduced germinability and increased number of abnormal seedlings (a seedling in a germination test that does not have the essential structures indicative of the ability to produce a normal plant under favourable conditions). Some of the well-known modern researchers like Roberts (1973c) and Powell (1988) also agree to the findings of Toole and his colleagues (1948).

Heydecker (1972) discussed the sequence of events in seed deterioration. According to him membrane damage is followed by impaired biosynthesis, which cause slower growth and a greater susceptibility to environmental stress resulting in

poor emergence potential, morphological aberrations and finally the loss of ability to germinate. This has been defined as physiological deterioration.

The causes of seed deterioration are not precisely known (Roberts, 1972). However, the possible causes of seed deterioration can be environmental, pathological or mechanical. These changes frequently occur in combination and act synergistically to enhance the process of seed deterioration. The alterations and abnormalities caused by these factors are associated with loss of viability.

Researchers believe that it is not possible to prevent the process of ageing, however, by proper storage its rate can be retarded and viability maintained for many years (Harrington & Douglas, 1970; Delouche, 1982; Gregg, 1982).

Copeland & McDonald (1985b) concluded that some of the important physiological symptoms of seed deterioration are (a) loss of enzymatic activity (b) reduced respiration (c) increase in seed leachates (d) increase in free fatty acid content.

Loss of seed viability and seed vigour are the most obvious consequences of seed deterioration. Therefore, it is important to discuss these terms separately.

2.2.1. Seed viability

Definition of the word viability varies with different people. Copeland & McDonald (1985a) reported that to the seed physiologist, a viable seed would be one whose radicle emerges through the seed coat during the process of germination.

However, the Association of Official Seed Analysts defined viability as "the emergence and development from the seed embryo of those essential structures which for the kind of seed in question show the ability to produce a normal plant under favourable conditions" (AOSA, 1983). On the other hand Ellis (1982) defined viability as "the ability of a seed to germinate and produce a satisfactory crop under

favourable conditions, provided any dormancy that may be present is removed". Ellis (1982) concluded that if a seed fails to germinate under optimum growing conditions not having dormancy, it should be considered non viable.

According to Abdul-Baki & Anderson (1972) as seeds age, they maintain germinability for some time. The seeds then enter a period of rapid decline in viability during which some seeds fail to germinate while others germinate and grow normally. Finally seeds exhibit total germination failure. The results of Kearns & Toole (1939) also suggested that there is a significant difference in quality among individual seeds within a seed lot. In other words when a seed lot as a whole deteriorates, those seeds that initially has the lowest quality lose viability first.

After studying barley and wheat seeds Abdul-Baki (1969) concluded that the time that marks the first detectable decline in germinability does not coincide with the actual beginning of deterioration. It was suggested that changes in the metabolic processes associated with seed deterioration may occur before a major decline in germinability is detected. This was illustrated by the great decline in synthesis of carbohydrates and proteins by seeds while their germinability still remained unchanged.

Kearns & Toole (1939) observed that deteriorated seeds, if they germinated often produced seedlings with sluggish growth. This means that during the process of seed deterioration the seed first loses the ability to produce normal seedlings, followed by a total germination failure. However, the results of Anderson (1970) indicated that reduction in seedling growth that precedes loss of germinability does not necessarily occur in every case of seed deterioration.

When seed deterioration proceeds far enough, the cellular systems that lead to the symptoms of deterioration are so disorganised that the seed loses the ability to germinate. Roberts (1984) called this ultimate catastrophe as viability loss or seed

death. Without a germination test it is not possible to designate a certain seed as dead or viable. Moreover, there is no sharp line of demarcation between life and death in seeds. Rather, seed death is a gradual and cumulative process. Woodstock (1973) reported that in seeds as they age more and more cells die until certain critical parts of the seed are unable to perform their essential functions.

Fortunately, in some crops, including soybean it is possible to predict percentage viability after a few months to many years over a wide range of environmental conditions (Ellis & Roberts, 1980a; Tekrony *et al.*, 1993).

Roberts (1973c) also stated that there was a great variation in the viability period of each individual seed. Moreover, it was concluded that the available tests of viability are all destructive. A seed that germinated during a germination test has given the indication that its life span may end some time in the future. Whereas the life span of those that failed to germinate had ended sometime before the start of the viability test. That is why Ellis (1982) concluded that it was difficult to define the life span of individual seed. However, Roberts (1973c) believe that the behaviour of populations of individuals can be defined very accurately.

2.2.2. Seed vigour

Seed vigour and loss of viability are interrelated. Factors that affect seed viability also affect its vigour. Viable seeds may not be vigorous or viability may be similar in two seed lots, but seed vigour may be lower in one seed lot than the other (Abdullah *et al.*, 1991).

There have been many attempts to agree upon a single all purpose definition of seed vigour. However, seed vigour is a multi-component concept. An agreed definition was not accepted until 1977, when the vigour committee of International Seed Testing Association defined seed vigour as "all those properties of seed that

determine the level of activity and performance of the seed lot during germination and seedling emergence".

McDonald (1980) defined vigour as those seed properties that determine the potential for rapid and uniform emergence over a range of field conditions. However, Woodstock (1973) defined vigour as "that condition of active good health and natural robustness in seeds which, upon planting, permits germination to proceed rapidly and to completion under a wide range of environmental conditions". However, farmers would like rapidity in growth and ability to survive under a wide range of field conditions to contribute to a profitable high yielding crop.

High seed vigour may show its effects in the shape of prolonged survival in storage, better emergence in the field, greater establishment of mature plants, and realisation of full yield potential (Heydecker, 1972). However, Woodstock & Freeley (1965) reported that before loss of germinability, the respiration level during the early stages of germination is closely related to subsequent seedling vigour in corn (*Zea mays*).

Pollock & Roos (1972) divided seed vigour into two components, genetic vigour and physiological vigour. Genetic vigour is seen in the difference of vigour between two genetic lines. Physiological vigour can be seen in the difference in vigour between two seed lots from the same genetic line. Seed physiologists normally deal with physiological vigour rather than genetic vigour.

Researchers agree that vigour measurements can be used for predicting potential performance in the field (Yaklich & Kulik, 1979; Yaklich *et al.*, 1979). Tiwari & Joshi (1989) reported that due to differences in vigour certain soybean cultivars exhibited poor emergence when planted deeply in the soil. It was observed that long hypocotyls and smaller seeds were a reliable criterion for the selection of high vigour soybean genotypes.

Abdul-Baki & Anderson (1973) compared high and low vigour soybean seed lots. It was concluded that vigorous seed lots of soybean absorb more sugars and amino acids from the imbibing media, incorporate these metabolites faster into polysaccharides and proteins and permit less leaching of unused metabolites into the surrounding aqueous media. Egli & Tekrony (1979) reported that a high vigour seed lot of soybean resulted in improved crop stands, but not necessarily high yield.

Harman *et al.* (1982) reported that vigorous seed lots were less susceptible to soaking injury. Their results showed that soybean or peas of poor quality produced more volatile aldehyde compounds during germination than highly vigorous seeds.

The speed of germination that has long been recognised as an indicator of seed vigour, is usually a more sensitive measure of seed deterioration than is loss of viability. This is supported by the findings of Toole *et al.* (1948) who observed that in aged vegetable seeds the radicle extension is delayed even before viability begins to decline.

Seed membranes play a vital role in the successful germination and establishment of seedlings and are a primary cause of differences in seed vigour (Powell, 1988). Seed ageing has been recognised as the major cause of reduced vigour and viability in many species. Ageing involve the process of seed deterioration. As mentioned earlier seed deterioration is the accumulation of irreversible degenerative changes until eventually the ability to germinate is lost (Powell, 1988).

There are different causes of why seeds deteriorate and lose their vigour and ability to germinate. Detailed consideration of the causes is beyond the scope of this thesis. A brief description is, however, necessary.

2.3. CAUSES OF SEED DETERIORATION

2.3.1. Depletion of food reserves

Depletion of food reserves was one of the earliest theories of loss in seed viability (Roos, 1984a). However, this theory has failed to survive wide criticism. James (1960) believes that most seeds contain enough food material to last thousands of years. Moreover, a cursory examination of just a few non viable seeds convinced Barton (1961) that there were plenty of food reserves left in the seed.

Roberts (1984) suggested that a decrease in protein and non reducing sugars, and increases in fatty acid and reducing sugars may be one of the possible causes of decline in seed viability. However, Copeland and McDonald (1985b) claim that the biochemical degradation processes in dry seeds are almost imperceptibly small and could not account for depleting the food reserves within the life span of most seeds.

As suggested by most research studies it is concluded that depletion of food reserves is not a possible cause of viability loss in seeds, however, poor mobilisation of food reserves during germination may well affect the vigour of the seedling.

2.3.2. Alteration of chemical composition

There is a strong support for the idea that food reserves mainly proteins may be altered chemically so that they are no longer available as energy sources. However, decreased solubility, partial breakdown and decreased digestibility have shown that proteins certainly undergo some kind of changes during storage (Barton, 1961). Roos (1984a) supported this idea. It was reported that many other food compounds (starches, lipids, vitamins) may change during storage. These changes may be qualitative or quantitative. Villiers & Edgcumbe (1975) reported that it was difficult to determine changes in biochemical parameters associated with loss of viability in dry seeds. On the other hand lipids in soybean were resistant to artificially enhanced

atmospheric oxidation if they remained within the structure of the seed (Priestley *et al.*, 1985).

2.3.3. Enzyme degradation and inactivation

Decline in enzyme activity is only a reflection of more basic changes in the enzymes themselves. Decreased activity of different enzymes such as catalase, dehydrogenase and decarboxylase, etc. in deteriorating seeds is well documented. Ching (1972) reported that ageing in quiescent seeds appears to be related to a gradual inactivation of the pre-existing enzymes and systems for protein and RNA synthesis. Protein synthesis plays an important role in germination, growth of the embryonic axis, synthesis of hydrolytic enzymes and other cellular machinery used for the mobilisation of storage reserves.

Halmer & Bewley (1984) also reported that the loss of viability is accompanied by an inability of the seed to synthesise protein upon imbibition. Decreased enzyme activity was thought to be responsible for this. Cherry & Skadsen (1984) considered an irreversible loss of certain biochemical events, for example the loss in synthesis of specific proteins similar to the viability loss in seeds. The general decrease in enzyme activity in the seeds lowers its respiratory potential, which in turn lowers energy (ATP) and food supply to the germinating seed (Copeland & McDonald, 1985b).

2.3.4. Genetic degradation

This theory is based on mutation that results in loss of the ability of the cell to duplicate and divide and thus grow. Abdalla & Roberts (1968) and Roberts (1972) reported that the more rapid the loss of viability during storage, the greater is the accumulation of chromosomal aberrations in the surviving seeds.

Roberts (1973a) placed seeds of beans, peas and barley in five different storage

treatments. As viability dropped a corresponding increase was noticed in the frequency of chromosome aberrations in the surviving seeds. It was concluded that although chromosomal aberrations are a good index of seed age, they are not the ultimate cause of seed deterioration. In another experiment Dourado & Roberts (1984) observed that even small losses of viability were associated with some genetic damage and no threshold losses occurred before chromosomal aberration.

2.3.5. Reduced respiration

The respiratory characteristics of deteriorated seeds during imbibition have received particular attention from investigators. Storage life is improved if the metabolic activities of the seeds are kept at minimum. According to Bramlage *et al.* (1978) reduced respiration at the start of germination is one of the important signs revealing that ageing has occurred in soybean seeds.

Respiration is a metabolic process by which a plant oxidises its food and provides energy for assimilation and breakdown of complex compounds. Moreover, respiration is a combined expression of the activity of a large group of enzymes that react together in breaking down food reserves (Copeland & McDonald, 1985b). As seeds continue to deteriorate, respiration becomes gradually weaker and ultimately leads to loss of germination. This means that alterations induced by ageing are ultimately revealed as metabolic deficiencies during germination.

Amable & Obendorf (1986) subjected soybean seeds to 4 controlled constant or fluctuating moisture content regimes at 25°C or 32°C to simulate the post-maturity preharvest environment. Seeds deteriorated rapidly under high moisture content regime at 32°C, and were non viable after 20 days mainly because of higher respiratory rates under applied conditions.

Veselova *et al.* (1988) reported that the delayed luminescence of air dried seeds

decreased with an increase in seed moisture content. Increase in delayed illuminations in germinating and swollen soybean seeds was caused by anoxia (lack of oxygen) under the seed coat.

2.3.6. Loss of membrane integrity

One of the most popular theories of seed deterioration relates loss of seed viability to loss or alteration in integrity of the seed membranes. In an experiment by Parrish & Leopold (1977) when dry soybean seeds were placed in water, solutes leaked out more rapidly from lower than high vigour seeds. Other researchers also believe that increase in solute leakage and loss of membrane integrity are important signs of ageing in seeds (Powell *et al.*, 1984; Schoettle & Leopold, 1984).

Powell & Matthews (1978) further reported that due to loss of membrane integrity a decline in the rate of food reserve transfer from the cotyledons to the growing axis caused a lower growth rate in pea seedlings. Moreover, Powell (1988) concluded that membrane deterioration is an early change in the process of ageing.

In an experiment by Schoettle & Leopold (1984) seeds were surface sterilised and dried before exposure to ageing treatment (40°C, and 100% relative humidity) for 2, 4 or 6 days. Solute leakage from imbibed soybean cotyledons increased with accelerated ageing. When hydration stress was minimised, a major contribution to the solutes leaking from soybeans after ageing was from cells that suffered massive membrane damage.

Parrish *et al.* (1982) and Parrish & Leopold (1978) believe that ageing of soybean seeds depressed the ability of their cotyledons to develop turgor. Turgor reductions were detectable immediately upon imbibition and evident after 2 days of ageing at a temperature of 41°C, and a relative humidity of 100%. Rowland & Gusta (1977) observed higher amount of seed leakage from low quality seeds of pea

(*Pisum sativum*) and faba beans (*Vicia faba*).

The dehydration of soybean seed after some critical stage perturbs the cellular membrane system, blocking cell elongation processes, such that they cannot resume upon imbibition (Senaratna & McKersie, 1983a).

Halmer & Bewley (1984) reported that during the first phase of imbibition membrane integrity is incomplete, however, with time the situation reverses, with the membranes either physically reverting to their most stable configuration or else being repaired by some mechanism. In non viable seeds or in seeds of low viability the repair mechanisms might be insufficient or the membrane disruption so extensive that repair is impossible.

There are innumerable symptoms of seed deterioration, but most of them appear to be a consequence of loss of membrane integrity, changes in the molecular structure of nucleic acids and reduction in enzyme activity. According to Roberts (1973c) these changes lead to reduced rate of germination, slow seedling growth, decreased ability to germinate under stressful conditions, increased probability of the development of morphologically abnormal seedlings and lower field emergence percentage.

Whatever the cause of seed deterioration is; membrane deterioration is an early change in the process of ageing. Powell (1988) support this hypothesis on the basis of (a) increased leakage of solutes from peas and soybean during the early stages of ageing; (b) vital staining of these seeds revealed that increase in solute leakage did not result from the development of dead tissues; (c) the uptake of Evans blue by the cells of soybean cotyledons; (d) reduced turgor shown by aged seeds during imbibition; and (e) increased leakage from seeds before viability decline.

The above review of literature indicate that there is enough evidence to reach a conclusion that loss of membrane integrity is a fundamental cause of seed deterioration.

2.4. FACTORS AFFECTING SEED QUALITY AND STORAGE LIFE

The rate of loss of viability in seeds depends upon the initial seed quality, conditions experienced by the seed on the mother plant, postharvest handling, storage conditions (Delouche, 1982; Gregg, 1982) and genetic constitution of the seed (Delouche & Baskin, 1973).

Environmental factors determine the rate of pathological and physiological seed deterioration. The following list shows some of the important factors that determine the storage life of the seeds and success during germination.

2.4.1. Preharvest environment.

2.4.2. Seed characteristics.

2.4.3. Postharvest handling.

2.4.4. Micro organisms.

2.4.5. Storage conditions.

2.4.1. Preharvest factors

Stresses to which seeds are exposed before and during harvest frequently have a marked influence on subsequent storability. However, Justice & Bass (1978) concluded that few, if any, of the studies reviewed were designed to test the relative effects of preharvest factors on seed vigour and viability. Field environment influences seed quality both before or after harvest maturity, however, much more information is available regarding field weathering following harvest maturity rather than before harvest.

The complex action and interaction of environmental factors frequently suppress the expression of genetic characters and cause the seed to exhibit additional traits attributed to the environment. These traits are visible in the shape of moldy and wrinkled seed coats, shrivelled seeds and necrotic areas on soybean cotyledons

(Andrews, 1982).

It is not easy to verify the effects of preharvest environment on viability and storage life of seed. Very little evidence is available to show what stages during growth and development are critical for various environmental factors to produce effects on seed viability (Austin, 1972).

The following are some of the important preharvest factors that can produce a prominent effect on subsequent seed vigour and viability during postharvest handling and storage.

2.4.1.1. Nutrition of the mother plant

A crop cannot produce high quality seeds unless it receives an optimum nutrient supply at the right time. Bagoury & Niyazi (1973) and Bagoury (1974) reported that the highest germination and lowest hard seed percentage in lentil was obtained when fertiliser levels were high. This indicated that undesirable hardseededness can be avoided by applying higher than recommended doses of fertilisers.

Mugnisjah & Nakamura (1984) reported that the application of a balanced dose of nitrogen and phosphorous to the mother plant at pod filling stage improves soybean seed quality.

Seedling vigour in wheat was significantly affected by seed weight and seed protein content (Bulisani & Warner, 1980). However, Jensen *et al.* (1972) found that most of the variations in three forage seedlings were due to the amount of nitrogen present in the seed, rather than the percentage protein or seed weight. Moreover, seed protein enhanced seedling vigour when nitrogen was withheld for more than the first 3 days of germination and apparently had no effect if adequate nitrogen was available during the early stages of germination.

2.4.1.2. Moisture stress

Water carries plant food nutrients from the soil to the plant and is vital in all other biochemical and physiological processes of the plant. In soybean moisture stress particularly at the critical stages of plant growth (flowering and pod filling) had an adverse effect on grain size, grain quality and grain yield (Doss *et al.*, 1974).

Continuous irrigation gave good quality and larger soybean seeds compared to plants subject to partial irrigation stress (Sumarno, 1986). Dornbos *et al.* (1989) studied the effect of drought stress on various aspects of soybean cultivar Gnome in a greenhouse experiment. It was found that as stress increased leaf resistance increased by 68%, transpiration decreased by 44%, and apparent photosynthesis decreased by 71%. Moreover, seed yield was reduced by 38 to 58%, primarily because fewer seeds were produced. Drought reduced standard germination percentage by 5 to 12%, and increased single seed conductivity by 19%. This suggested that not having other stress conditions high quality seeds can be produced by studying long term climatological records, careful selection of planting date and cultivar, to avoid water stress, particularly at the flowering and pod filling stage and to supply additional water by irrigation, if required.

2.4.1.3. Field weathering after physiological maturity

Temperature controls the growth rate of plants and determines seed quality, especially under high relative humidity conditions. Repeated wetting and drying of mature soybean seeds in pods before harvest caused poor laboratory germination and under extremely moist conditions seeds germinated within the pods (Banamurthy & Gupta, 1981). Green *et al.* (1966) reported similar results. Rubel *et al.* (1972) also observed that 24 to 40 days after flowering, temperature played a key role in determining the fatty acid composition of oil and the final oil percentage of soybean seed.

After exposure to alternate wetting and drying, high temperature and high relative humidity after physiological maturity before harvest and mechanical damage during harvesting, threshing and processing soybean seeds did not store well (Gupta, 1976).

Dassou & Kueneman (1984) studied thirty five soybean genotypes and reported that after 1 h of soaking hard seeds ranged from 0 to 64%. Hard seeds had significant correlation with seedling emergence after incubator weathering. Large seeded genotypes were found susceptible to field weathering. Some small seeded genotypes were resistant; others were susceptible. Black seeded genotypes were more resistant to incubator weathering than yellow seeded genotypes. Genotypes with a high percentage of seedling emergence after incubator weathering also had high seedling emergence after ambient storage.

There is some evidence that the position of the seed on the mother plant has a significant and constant influence on seed vigour and viability in soybean. For example Keigley & Mullen (1986) reported that seeds of pods collected from the middle part of the plant had superior germination than those collected from the lower or upper half of the plant.

2.4.2. Seed characteristics

The genetic constitution of the seed plays a vital role in determining its storage life (Mayer & Mayber, 1963; Ellis *et al.*, 1982). For example James (1967) discovered significant differences in the storage life of various cultivars of cucumber (*Cucumis sativus*), bean (*Psophocarpus tetragonolobus*), peas (*Pisum sativum*), sweet corn (*Zea mays*) and water melon (*Cucumis citrullus*).

Burgess (1938) studied eight soybean cultivars and noticed germination variations between 21 to 99% after 4 years compared to between 95 to 99% variations after 5 months storage. These variations were attributed to differences in seed

characteristics.

Neal & Davis (1956) reported that some inbred lines of maize (*Zea mays*) maintained viability better than others although the differences among cultivars were not revealed until the third and fourth year of storage. In one experiment by Roos (1984b) conducted on eight varieties of mungbean (*Phaseolus mungo*) different cultivars deteriorated at different rates. This behaviour was attributed to the differences between cultivars in their seed characteristics.

According to one report the inherent short life span is more a characteristic of seeds of modern soybean cultivars, rather than the species as a whole, however, considerable variation exists in seed longevity within the species as well (Lassim & Delouche, 1981). Delouche *et al.* (1973) also reported that due to apparent differences in seed characteristics (seed size, seed coat, seed colour) some soybean seeds were inherently short lived compared to others.

Singh *et al.* (1986) studied the inheritance of seed storability in crosses of soybean cultivars Bragg x T49, and Kalitur x Alankar. It was observed that seed genotype had little effect on seed storability. Seeds from F_1 plants showed storability similar to that of the better parent. The back cross breeding procedure was recommended for transferring high seed storability genes to high yielding cultivars. This may lead to improved prospects for soybean cultivation in the tropics.

The following are some of the important seed characteristics that can affect storage life and are determined by the synergistic effect of genetic and environmental factors.

2.4.2.1. Seed coat

The seed coat plays an important role in regulating the exchange of water, nutrients and gases between the seed and outside atmosphere (McDonald *et al.*, 1988a).

Calero *et al.* (1981) studied the mechanism of water absorption (the uptake of

water into the tissues of seed) in different soybean cultivars and observed that small seeds had a higher percentage of seed coat by weight whereas larger and medium seeds had a lower percentage of seed coat. Ragus (1987) also agrees to these findings. This indicated that a higher percentage of seed coat by weight may be associated with thicker seed coats and improved storability. Kuo (1989) concluded that in soybean, delayed permeability of the seed coat is a promising character for the selection of high quality seed lots that possess good storage life. Yaklich *et al.* (1986) also suggested that seed coat versus embryo ratios can be related to seed coat permeability or storage life of the seeds.

Larson (1968) obtained poor storage, rapid imbibition and greater seed injury if the seed coat was removed from pea (*Pisum sativum*) seeds, indicating that the seed coat plays a vital role in storage and during germination. These findings are further supported by Powell & Matthews (1978) who observed that cracks in the testa of pea seeds encouraged rapid water uptake, cell death, solute leakage and resulted in poor vigour.

Hill *et al.* (1986a) reported that ruptured seed coats in soybean were caused by the cotyledons expanding against the seed coat. The incidence of ruptured seed coats was positively associated with seed weight, seed volume and seed width. Moreover, high soil moisture availability during seed filling reduces the number of impermeable seeds by disrupting seed coat integrity. This suggested that moisture supply at seed filling can be exploited if tough seed coats are desired for better storage life.

The proportion of impermeable seeds at harvest ranged from 12.5% in soybean variety Bragg to 80% in case of EC-39140 and decreased to a constant level after ten months (Shahi *et al.*, 1982). A tendency of the seed coat towards impermeability contributes to hardseededness and thus better storability.

2.4.2.2. Hardseededness

Hardseededness is potentially a valuable trait for reducing the effects of field weathering on soybeans (Hinson and Hartwig, 1982). This indicated that hardseededness reduces seed moisture fluctuations which can occur with alternate wetting and drying in the field. Potts *et al.* (1978) found that seeds of a hard seeded soybean genotype maintained 80 percent viability for up to nine weeks following maturity; whereas an adapted variety grown under identical conditions maintained 80% viability for only 3 to 4 weeks. The impermeability of the seed coat to water was identified as a natural barrier to one of the primary factors affecting viability or storage life of soybean seeds. It was reported that the idea of hardseededness could be beneficial to the retention of seed viability under tropical and subtropical storage conditions.

According to Hinson & Hartwig (1982) individual seeds in a soybean seed lot vary in the degree of hardseededness and the rate of imbibition. This means that mature un-harvested seeds with hard seed coats may undergo less swelling and shrinkage as they are likely to absorb less moisture from light rains and heavy dews. Therefore, hardseededness encourages good storage life as they experience less field deterioration (Tekrony & Egli, 1977) and reduced uptake of moisture during storage at high relative humidity (Potts *et al.*, 1978).

The above researchers have suggested hardseededness as a possible remedy to seed viability problems in the tropics. However, Hinson and Hartwig (1982) wonder that soybean seeds with hard seed coats will require scarification before planting if germination has to occur uniformly. Therefore, more research is needed to evaluate this potential trait.

2.4.2.3. Seed size

Seed size also plays an important role in determining seed vigour and viability. Vanangamudi (1988) observed that smaller soybean seeds retained their viability better than larger seeds. Improved storability in case of smaller seeds was attributed to better seed coat structure (thicker and fine seed coats).

Justice & Bass (1978) concluded that heavy, mature seeds are superior to light immature seeds as far as performance during storage and germination are concerned. However, it was not clear that the differences in seed storability and vigour were due to differences in seed size or whether factors such as seed coat and pre-storage history were also responsible.

Research workers have obtained contrasting effects of seed size on germinability. For example, Armstrong *et al.* (1988) reported a greater percentage emergence, rate of emergence and shoot or root fresh weight accumulation in a soybean cultivar with large seeds than ungraded or small seeds. However, Edward & Hartwig (1971) observed that small and medium seeded soybean cultivars were superior in rate of emergence and root development. Paschal & Ellis (1978) also obtained the best field emergence from soybean cultivars with the smaller seed size. In another study conducted by Carlton & Cooper (1972) large seeded cultivars of three forage legumes exhibited greater overall emergence percentage.

Jensen *et al.* (1972) while evaluating seedlings of various forage legumes, reported a positive correlation between seed weight and the force exerted during seedling emergence. Cultivars with higher seed weight exerted greater emergence force. Townsend (1972) reported similar results. Abdullahi & Vanderlip (1972) compared the performance of different seed lots of sorghum both in the field and under laboratory conditions. It was found that establishment was highest from medium size seeds followed by larger seeds.

Black (1958) separated seeds of subterranean clover (*Trifolium subterraneum*) by size to find out if larger seeds would produce seedlings of higher vigour compared to smaller seeds. When seeds of uniform size were planted at equal plant densities, the number of plants surviving decreased throughout the growing season as competition between plants eliminated part of the population. In uniform planting, there was no difference between larger and smaller seeds in the number of dead seeds. However, when larger and smaller seeds were planted together, the number of plants from the larger seeds remained approximately constant, whereas two-third of the plants from small seeds were eliminated by competition.

By contrast Williams *et al.* (1968) studied a mixed community of *Trifolium incarnatum* L., and *Trifolium subterraneum* L., planted with seeds of different sizes. They reported that no plant elimination occurred due to competition. However, it was observed that *Trifolium subterraneum* repressed the development of *Trifolium incarnatum* except when the smallest seeds of the former species were mixed with the largest seeds of the later specie.

Hill *et al.* (1986b) separated nine soybean lines into three size classes based on seed diameter. Seed permeability was determined by soaking in water for 2, 4, 8, 24 or 72 h. Smaller seeds exhibited the impermeable response at a higher frequency than larger seeds. Differences in seed permeability among size classes increased with soaking time.

2.4.2.4. Seed colour

Dharmalingam & Basu (1987) observed significant differences in field emergence in mungbean (*Phaseolus mungo*) varieties of different seed colour. A single recessive gene was thought to control hardseededness in soybean. Maryushkin *et al.* (1987) linked hardseededness with black seed coat colour.

Great variation in resistance to field weathering (Mugnisjah *et al.*, 1987) and tolerance to high temperature during storage and germination (Emerson & Minor, 1979) exists among soybean cultivars having different seed coat colour. A black seeded isoline of soybean cultivar IAC-8 stored better and had a significantly higher seedling emergence than a yellow seeded isoline (Kueneman & Costa, 1987). Koslanund & Delouche (1987) reported poor storability and reduced germination in soybean seeds with light coloured seed coats.

2.4.3. Harvesting and postharvest handling

After maturity, seeds pass through a number of processes including harvesting, drying, cleaning, grading, transportation and seed storage. Mature good quality seeds that go through proper storage produce desired crop stands under optimum field conditions. However, unavailability of good quality seed, proper storage and unfavourable field environment lead to poor viability (Gregg, 1982).

2.4.3.1. Harvesting

Harvest losses and postharvest handling are regarded as one of the most critical stages of crop production. According to Hepperly *et al.* (1982) most of the problems of seed storage begin with mechanical damage to the seed during harvesting and postharvest handling. They reported that the moisture content of the seeds may be so high that heating occurs before seeds are dried to a safe moisture content or it may be so low that the seeds suffer impaction damage on handling.

The system of harvesting varies from hand harvesting in the potential soybean growing tropical and subtropical countries such as Pakistan and India to sophisticated machine harvesting methods as in the United States of America (Tedia, 1982). Harvesting that leads to mechanical damage should be avoided. Soybean harvest losses can be reduced by harvesting at moisture levels too high for safe storage

(Tedia, 1982) but a delay in harvest to achieve lower seed moisture contents aggravated threshing losses in soybean (Tanner & Hume, 1978).

It has been reported by Green *et al.* (1966) that hand and machine harvested lots in soybean harvested on the same day produced higher viability from hand harvested seed lots.

2.4.3.2. Threshing

Research work on seed injury expanded greatly during 1930's, with the arrival of combine threshers (Justice & Bass, 1978). Due to their unique seed structure and vulnerability to mechanical damage some crop species, including soybean, received greater attention than others. The problem of mechanical damage to seed during postharvest handling was particularly severe in soybean (Tedia, 1982) especially in case of large seeded cultivars (Green *et al.*, 1966). Mechanical injury during threshing was responsible for a reduction in the vitality and storage life of soybean in a study conducted by Oatout (1928).

In an experiment by Bartsch *et al.* (1986) seeds of soybean cultivars Williams and Amsoy-71 at 8, 13 or 18% moisture content were impacted at a velocity of 5, 10 and 15 metres per second. The resulting damage was measured with tetrazolium chloride topographic staining test. Soybeans at 18 and 13% moisture content exhibited similar impact damage resistance. It was observed that impaction damage increased significantly as seed moisture content dropped from 13 to 8%. Impaction treatments of 15 metre per second to seeds at 8% moisture content produced seed fracture. Direct impact to the radicle produced the largest reduction in seed germination and vigour.

Etched seeds (seeds with a crack in cotyledons) had a lower germination and were more susceptible to damage from low drop heights as compared to the non etched

seeds (Burchett *et al.*, 1985). According to Singh & Seitia (1974) smooth seeded soybean varieties maintained viability longer than wrinkled seeds.

2.4.3.3. Seed drying

The ways seed may be dried include sun drying, natural air drying, dehumidified air drying, drying in storage and use of desiccants in sealed containers. Adams *et al.* (1983) reported that soybean seeds shelled and dried outside the pod lost moisture more rapidly and had poorer viability than seeds dried slowly within intact pods. Due to ultraviolet radiation, intense direct sunlight was also considered to be harmful to seed viability (Harrington & Douglas, 1970).

Hot air caused seed cracking because drying occurred very rapidly. For soybean air drying at a temperature of 38°C, and a relative humidity of between 40% and 50% was recommended (Tanner & Hume, 1978). Traditional drying in the tropical and subtropical countries do not reduce seed moisture adequately which normally activates seed deterioration (Gregg, 1982). Delouche (1974) recommended that after harvest soybean seeds should be dried as follows:

1. From 12 to 13% moisture in temperate climates and stored in a dry ventilated warehouse.

2. To 9% moisture (60% relative humidity) or less in tropical and subtropical climates and stored in conditioned storage facilities at 20°C to 25°C, or less.

3. To 9% or less moisture in tropical and subtropical climate and sealed in vapour proof packages, such as heat sealed polythene bags.

The above recommendations are applicable to seed storage of between 8 to 10 months.

2.4.4. Microorganisms

Plant physiologists are interested in, (a) physiological and biochemical changes that occur during seed deterioration, and (b) how these changes contribute to the process of seed deterioration. On the other hand plant pathologists tend to be more interested primarily in the microorganisms rather than in the biochemical process of seed deterioration (Halloin, 1984).

Kueneman (1982) concluded that plant pathogens play an important role in the process of seed deterioration both before and after harvest. Moreover, the effects of plant pathogens during seed storage are great, but secondary to the deleterious physiological and biochemical changes that occur during ageing.

Sinclair (1982) reported that the conditions that favoured rapid seed deterioration also favoured microbial growth, it was difficult to identify the aspects of deterioration induced by micro organisms. Furthermore, there is increasing evidence from the field and histo-pathological studies that multiple as well as single fungal infections occur in soybean seed and contribute to poor quality.

Most of the destructive pathogens of soybean are seed-borne. Sinclair (1978) reported that almost 66 fungi, 6 bacteria and 8 viruses are associated with soybean seeds in the tropics and subtropics. According to other reports such as Ellis *et al.* (1977) and Tenne *et al.* (1977) high temperature and moisture condition in tropical and subtropical regions accelerate physiological and pathological deterioration in soybean seeds.

Halloin (1984) suggested that most of the pathological studies investigate seed borne diseases and their control rather than the possible contribution of pathogens to the process of seed deterioration. Very little can, therefore, be expected from plant pathologists as far as the role of microorganisms in seed deterioration is concerned.

Seeds are attacked by two types of fungi. Field fungi need free water and high

relative humidity for infection and development, whereas storage fungi usually infect seeds in storage and do not occur at a high incidence on seeds before harvest (Hepperly *et al.*, 1982). Fungi that frequently attack soybean seed during or after ripening in the field include genera *Fusarium, Alternaria, Helminthosporium and Cladosporium* (Garzonio & McGee, 1983) whereas storage fungi include genera *Aspergillus* and *Pencillium* (Halloin, 1975). Harman (1983) reported that storage fungi reduced the storage life of soybean, particularly when the seeds were stored in equilibrium with a relative humidity of 68%. Field fungi remained viable but quiescent during storage, however, they were capable to restart their growth when the moisture contents of the seeds increased.

Harman & Drury (1973) reported that mitochondria from pea seeds infected with *Aspergillus ruber* were less active as compared to healthy seeds. Harman & Nash (1972) reported the production of a toxin by *Aspergillus ruber* that killed the embryonic axis of pea seeds before actual infection by the fungus.

Yaklich & Kulik (1987) observed more infection of soybean seeds during incubator ageing and significantly less infection in cultivars that possessed impermeable seed coats. An experiment performed by Naudi *et al.* (1982) revealed that natural storage of oil seeds resulted in an increased seed infection and decreased germination percentage. High relative humidity with high temperature escalated the problem. Delay in harvest decreased germination percentage of soybean and increased fungal infection under tropical conditions (Paschal & Ellis, 1978).

Soybean seeds that had been injured in the field by insects, pests or diseases were more vulnerable to rapid seed deterioration in storage (Sirising & Kogan, 1982). This was further confirmed by Hilty & Goodfellow (1988) who observed a significant reduction in soybean germination when seeds were treated with dilution of *Phomopsis phaseoli*.

A great deal of literature supports the idea that the incidence of most of the common diseases enhances the process of seed deterioration. Moreover, the rate of seed deterioration is higher at high seed moisture levels particularly when accompanied by high temperature. These ideal conditions for the growth of pathogens are met in the tropical and subtropical regions (Ellis *et al.*, 1977; Tenne *et al.*, 1977; Sinclair, 1978).

The two major ways by which microorganisms damage seeds are (a) production of enzymes, i.e. cellulases, pectinases, amylases, lipases, proteases and nucleases etc. that disturb the physiological and biochemical events occurring in the seeds (Halloin, 1975) and (b) toxins that damage cell membranes (Harman & Garnett, 1972) and cause elongation of cell and necrosis of the embryonic axis (Harman, 1973). All these changes ultimately result in a loss of seed vigour and viability.

It is concluded that the role of fungi and other microorganisms in seed deterioration needs a common understanding of seed physiologists and seed pathologists.

2.4.5. Storage conditions

The purpose of seed storage is to maintain high seed quality, good potential for planting and the best possible physiological state. Delouche & Baskin (1973) concluded that planting potential is largely determined by inheritance, initial seed quality, pre-storage handling, length of storage and storage environment.

Justice & Bass (1978) suggested that desirable candidates for storage are mature seeds of normal size and appearance that are relatively free of mechanical injuries and storage microorganisms. An immature seed, a seed with an unbalanced chemical composition or one mechanically damaged, permitting early entry of micro organisms, would be at a disadvantage during storage.

Soybean is a notorious orthodox (seeds that can be dried to very low moisture content without noticeable loss of vigour and viability) as far as seed storage is concerned. Gopa & Mukherji (1986) reported that loss of germination in soybean at 100% relative humidity and 40°C, was more rapid compared to maize and mustard. This can be attributed to the fact that the growing point of the embryonic axis (meristems of radicle and plumule) of soybean seed is highly vulnerable to ageing, and therefore should be considered vital as far as the maintenance of viability is concerned (Chauhan, 1985).

Fortunately, under proper storage conditions maintenance of soybean seed viability for longer duration is possible without any significant effect on crop yield (Ricker, 1980).

From the above reported evidence it is concluded that two environmental influences, relative humidity and temperature, are critical in determining storage life of seeds. The effects of a third factor, the gaseous environment (amount of O_2 in the air) in which seeds are stored, is far less well understood, however, most of the reports have suggested that generally a decrease in oxygen concentration around the seeds improve storage life.

2.4.5.1. Seed moisture, temperature and ambient oxygen

In orthodox seeds decreasing either seed moisture, storage temperature or oxygen increases the storage life of seeds. The results of Roberts & Abdalla (1968) illustrate this point. These researchers have reported that higher seed moisture, temperature and ambient oxygen enable the seeds to respire at a higher and thus the seeds deteriorate at a higher rate.

The deleterious effect of oxygen on broad beans (*Phaseolus vulgaris*), barley (*Hordeum vulgare*) and peas were more pronounced at higher moisture content.

Contrary to this Ohlrogge & Kernan (1982) reported that at high temperature and high relative humidity (44°C, 100% relative humidity) oxygen concentration did not show effect on seed longevity.

The moisture content of the seeds is a function of the relative humidity of the air surrounding the seed. However, at a given relative humidity seeds may reach two different equilibrium moisture contents; one by increasing the relative humidity from a low level and another by decreasing the relative humidity from a high level (hysteresis effect). Hysteresis is defined as the difference in the amount of water involved during adsorption (the taking up of one substance at the surface of another) and desorption (reverse of adsorption). The higher desorption isotherm has been attributed to the appearance of additional points of attachment (polar sites) for bound water as a result of tissue swelling (Abdul-Baki & Anderson, 1970).

Most crop seeds lose viability rapidly at relative humidity approaching 80% and temperatures of 25°C to 30°C, but can be kept ten years or longer at relative humidity of 50% or less and a temperature of 5°C, or lower (Toole, 1950). Harrington (1960) reported that for safe storage the sum of percentage relative humidity plus the temperature in °F should not exceed 100.

In an experiment by Dorworth & Christensen (1968) the effects of moisture content, temperature and storage duration on germination of soybean seeds were studied. The results showed that various combinations of temperatures and seed moisture content commonly found under tropical conditions were conducive to rapid loss of soybean viability. They suggested that either low temperature or low seed moisture content is essential for the maintenance of seed viability and that both may be needed.

Oatout (1928) reported that high temperature and lack of ventilation resulted in a loss of viability in soybean seeds having moisture content above 14%. Barton (1961)

reported that seed moisture content was one of the most important factor influencing the storage potential of seeds. Moreover, great variation in the time required for the seed to reach equilibrium with the surrounding environment exist among different species and even among different cultivars of the same species. However, under normal storage conditions the moisture content of the seed reaches equilibrium with the surrounding air, if given enough time.

For each 1% reduction in seed moisture content and for each 5°C decrease in storage temperature the life span of the seed is doubled (Harrington, 1960). However, Roberts (1984) clarified that Harrington's rule was applicable between a seed moisture content of 5 to 14% and storage temperature of 15°C to 50°C. Harrington & Douglas (1970) reported that seeds at 14% moisture content are in equilibrium with 70% relative humidity. The seeds lose moisture at 45% relative humidity and gains moisture at 85% relative humidity.

For longer storage life it is therefore important to keep the physiological activity of the seed to a minimum. This can be achieved by providing conditions to keep the seeds at their optimum moisture content accompanied by low temperature and low ambient oxygen concentration (Owen, 1956).

Soybean cultivar Akita-daizu was stored at a seed moisture content of 6 to 12% under controlled conditions at 26°C and 80% relative humidity. Samples drawn at intervals of two months between 2 to 18 months showed a decrease in seedling growth rate and hypocotyl respiration with increase in storage period and seed moisture content (Sripichitt *et al.*, 1989).

2.4.5.2. Storage containers

Storage containers play a vital in extending seed storage life which cannot be over emphasised. In one experiment soybean seed were stored at different seed moisture

contents in 0.5 mm thick polythene bags derived from imported urea fertiliser bags and were assessed for germination percentage, root growth and shoot growth. Seed viability was satisfactory after 12 months storage at 10°C or at room temperature (20°C to 25°C). Cold storage for 4 to 7 months increased seedling root length, but decreased stem length compared with seedlings grown from seeds stored at room temperature (Majid *et al.*, 1982). In another experiment by Taoi *et al.* (1986) conditioned storage (10°C, 55% relative humidity) was superior followed by nitrogen gas storage (100% nitrogen gas flushed at 130 ml per minute flow rate on seeds stored in 40 liire milk container at 25°C) then storage in storenouse.

Arulnandhy & Senanayake (1988) and Cooper (1959) reported that storage at lower seed moisture contents in polythene bags was useful in maintaining seed viability in soybean. According to Ellis & Roberts (1982) pea seeds dried to low moisture content (7%) showed extended longevity in hermetic storage.

Vanangamudi (1988) reported that soybean seeds stored in aluminium foil and polythene laminated pouches retained higher vigour and viability than those stored in cloth bags. Ayub & Sherin (1987) studied the effect of storage container on the viability and vigour of soybean at five locations in Pakistan. Seeds stored at lower moisture contents in plastic bags maintained high viability and maximum seedling vigour. Hepperly *et al.* (1982) reported an improvement in storage life of soybean seeds submerged in oil especially when ambient temperature and relative humidity were high.

2.5. CHEMICAL SEED TREATMENTS

It is always beneficial to use high quality, disease free seed for planting. However, at times seeds have a high incidence of seed borne fungi. Therefore, chemical seed treatment may be beneficial in improving germination of poor quality seeds.

In a seed treatment experiment soybean seed of cultivar JS 72-44 were stored from in gunny bags, cloth bags or in tins after seed treatment with fungicides Dithane M-45, thiram and Bavistin, thiram and captan or thiram alone. All the treatments maintained high germination in fresh seeds from 70.5% in the control to 88.9 to 98.8% with Dithane M-45. Dithane M-45 was the most effective. Seed treatment reduced post-emergence mortality.

Gorecki & Harman (1987) reported improved viability when antioxidants as tocopherol and BHT were applied to the seeds.

Miles (1987) reported an increase in the standard germination and accelerated ageing test results of soybean seeds infected with *Phomopsis species*. Wall *et al.* (1983) reported that the germination percentages of soybean cultivars Cumberland, Wells and Williams were significantly improved when damaged and poor quality seeds or seeds infected with *Phomopsis* were treated with captan or thiram. Germination was improved by treating the seeds with captan or thiram, however, they did not observe any significant field emergence differences between seeds treated with captan or thiram.

Petruzzelli & Taranto (1985) reported that the application of gibberellic acid and ethephon (dissolved in acetone) were effective in retarding the rate of seed deterioration in wheat (*Triticum aestivum*) during storage. In one of the experiment by Dey & Mukherji (1984) 2 months old seeds of soybean (*Glycine max* L. Merrill) and 4 months old seeds of sunflower (*Helianthus annuus*) were treated with a dry dressing of iodine in a calcium carbonate carrier. Both these treatments effectively reduced seed deterioration during subsequent storage under elevated temperature and different relative humidity regimes.

Edje & Burris (1971) reported a significant increase in the emergence percentage and survival of low vigour soybean seeds when treated with captan. Seed treatment is

not always effective. For example red earth treatment of soybean seeds reduced field emergence and increased the percentage of abnormal seedlings (Vanangamudi & Karivaratnaraju, 1987).

Pyndji *et al.* (1987) used refined maize oil, oil palm, soybean oil and sunflower oil as a medium for heating soybean seeds to control some seed borne fungi. Infected seeds of five cultivars ranging in age from 6 months to 24 months were treated in one of the heated oils for 2 minutes to 15 minutes at 27°C or 32°C. All treatments checked fungal attack. Heat treatment was more effective in seeds less than 1 year old compared to seeds that were more than 1 year old.

Gupta & Chatrath (1983) reported maximum degradation of thiram when seeds were stored in cloth bags, followed by paper and alkathene lined jute bags. Though storage at different temperatures (10°C to 35°C) had no effect; interactions between the different factors of temperature, period of storage and type of container were significant. Ferriss *et al.* (1987) reported improved performance of both high and low quality seed lots in the field when treated with thiram at the time of cleaning.

It is concluded that soybean seeds are basically short lived. They require a special storage environment. In store, soybean is part of a dynamic system, including the microorganisms, insects and physical factors like humidity, temperature and the surrounding gaseous environment. Therefore, the ultimate objective of seed storage should be to provide such an environment that keeps the physiological and pathological activities of the seed at a minimum.

2.6. PHYSIOLOGICAL SEED TREATMENTS

Physiological treatments are applied to enable the seeds to express their maximum potential during the process of germination and seedling emergence. These treatments are beneficial to soybean seeds especially to seed lots that have low initial

vigour. This is because soybean seeds absorb water as efficiently as other common legumes and cereals, but the rapid rate of imbibition is a major factor responsible for soaking injury in soybeans (Parrish & Leopold, 1977).

Waggoner & Parlange (1976) reported that the initial period of rapid water uptake lasted about 30 minutes in peas, but only 5 to 10 minutes in soybean. This initial inrush of water into the soybean cotyledons was so rapid that Parrish & Leopold (1977) regarded it to be responsible for a major part of soaking injury.

Duan *et al.* (1987) reported that the moisture absorbing capacity of soybean seeds is positively correlated with protein and negatively correlated with the oil content of the seed. This suggested that being high in protein also makes soybean an efficient water absorber and thus more liable to water uptake injury. Seeds particularly those of low vigour thus need to be prepared for germination. In the following discussion reference has been made to some of the physiological seed treatments that improve germinability of seeds if properly applied.

Mukherji & Dey (1985) soaked soybean seeds for a brief period followed by drying back, but failed to improve its germinability due to rapid imbibition damage. However, osmotic control by 2 step hydration (osmotic treatment with polyethylene glycol, then soaking in water) followed by drying improved immediate germination, whereas 3 step hydration (moisture equilibration before osmotic treatment and soaking in water) followed by drying had a positive effect on storability. In case of dehumidified seeds, soaking followed by drying was promising.

Seong *et al.* (1988) tested germination and seedling length of untreated and osmo-conditioned (PEG 8000) seeds of soybeans cultivar Williams after preconditioning at 15°C or 30°C to 30 or 50% seed moisture content for 2 to 8 days. Preconditioning of untreated seeds at 30°C, for 2 days accelerated seedling growth at both moisture levels, but seedling growth was reduced by longer periods of preconditioning and by

osmo-conditioning.

Soybean seeds are very vulnerable to damage from drying back after long hours of imbibition. This was shown in an experiment performed by Senaratna & McKersie (1983a) where the seeds were tolerant of drying to 10% moisture content after 6 h of imbibition, but drying after 36 h led to loss of germination, increased seedling abnormalities and electrolyte leakage from isolated embryo axes. Galbraith *et al.* (1988) reported that soybean seeds germinated at 22°C, in uniconazol for 72 h and then subject to a temperature of 50°C, for 2 h exhibited 26% less solute leakage, 22% less Sodium (Na) leakage and 20% less Potassium (K) leakage than the controls.

Tilden & West (1985) reported a reversal of the effect of ageing by slowly imbibing and then redrying soybean seeds. They observed reduced electrolyte leakage and increased seed vigour after drying back treatment. The results provided evidence for the metabolic repair of plasma membranes and other sub-cellular components when the seeds were imbibed at a slow rate. This means that the beneficial effects of hydration are evident if soybean seeds are dried back slowly. However, if the rate of imbibition and drying back is high then the seeds are exposed to severe water uptake injury.

The beneficial effect of hydration and a drying back treatment in soybean has also been confirmed by Saha & Basu (1982). To avoid soaking injury low vigour soybean seeds were preconditioned to moisture equilibration with a water saturated atmosphere or soaking in 40% polyethylene glycol (PEG 6000) for 24 h and kept in contact with moist sand for 16 h, then dried back. This resulted in improved germination. Seeds pre-imbibed in an osmoticum are reported to show improved emergence if drying back is avoided. Soaking in polyethylene glycol improved initial emergence, but soaking in water reduced initial emergence (Helsel *et al.*, 1986). This may indicate that the deterioration of soybean seeds predisposes the embryonic axis to injury during the initial period of imbibition.

Woodstock & Taoi (1981) prevented soaking injury in low vigour seeds when excised axes were imbibed on blotters containing 30% polyethylene glycol. These studies indicated that seed deterioration decreased the ability of the seed axes to tolerate rapid water uptake at the start of imbibition. This weakness may be compensated by osmotic control of water uptake. These results are further supported by Woodstock & Taylorson (1981). Their findings indicated that slow imbibition in polyethylene glycol had a vital role in avoiding water uptake injury in soybean seeds.

Senaratna & McKersie (1983b) placed dry soybeans in water and reported reduced germination as a result of imbibition injury. That is why Ellis (1982) recommended that very dry seeds of most of the legumes should be conditioned in high relative humidity before testing for viability. This recommendation was supported by Saha & Basu (1984) who controlled soaking injury in soybean seeds by providing an atmosphere of 100% relative humidity at 28°C for 24 h.

Tilden & West (1985) observed a 10% increase in the germinability of aged soybean seeds and decrease in the leakage of electrolytes by controlling the rate of imbibition. McDonald *et al.* (1988a) studied soybean seed imbibition and absorption of water by various seed components. After 72 h imbibition, the embryonic axis was the most hydrated portion of the seed. The cotyledons and embryonic axes of aged seeds did not differ in moisture uptake compared to non deteriorated seeds. The axes from aged seeds, however, absorbed less water than the unaged controls.

Seed treatment with captan or linseed oil improved seed resistance to dry soils and to flooding. The adverse effects of a 12 h flood decreased as the time from sowing to flooding decreased or as seed moisture content increased (Peske, 1983). Ikeda (1985) compared the performance of pre-germinated seeds with direct sown seed and observed an increase in the percentage of seedling establishment in pre-germinated seeds of soybean for sowing at a depth of 1 to 3 cm.

Schultz & Evenson (1983) observed a 3.6% increase in germination of pre-hydrated seeds compared with non pre-hydrated seed. Response to pre-hydration decreased as mechanical damage levels (measured as seed coat damage after soaking for 5 minutes in 1% hypochlorite solution) increased. Seed water content increased during pre-hydration at the rate of 0.2% per hour, compared with 4.5% per hour for non pre-hydrated seeds. It was suggested that pre-hydration of soybean seeds before germination could reduce or prevent imbibition injury.

Das *et al.* (1989) soaked rice (*Oryza sativa*) seeds in 25% polyethylene glycol (PEG) and reported an increasing effect on plant height, tiller number, shoots dry weight and leaf area

Besides its use in the evaluation of water uptake injury in soybean seeds, polyethylene glycol has a great potential in screening cultivars for improved emergence under moisture stress conditions (Seong *et al.*, 1988).

2.7. PLANTING CONDITIONS

The germination capacity of a seed lot is the number of seeds that are capable of establishment into healthy fully autotrophic seedlings such that they form a satisfactory crop under defined field conditions (Bekendam, 1982).

Even if laboratory germination of a seed lot is high, its success under field conditions depends on the environment experienced by the seeds after sowing. Soybean seeds lack a specific light requirement for germination, but are no less dependent than other seeds on optimum moisture, temperature and aeration (Howell, 1963).

2.7.1. Soil and seed, moisture and germination temperature

Major constraints in soybean production are associated with its germination and

emergence that are greatly affected by soil moisture and temperature. Hobbs & Obendorf (1972) reported that soybean seeds soaked at low initial moisture content led to the formation of transverse cracks, browning of cotyledons and produced poor germination capacity.

Soybean seeds require more water for germination and seedling growth than maize or sunflowers (Seong, 1986). However, the seeds of soybean obtained sufficient water for germination from a soil that was too dry for the germination of maize and sugar cane (Hunter & Erickson, 1952).

Oliveira *et al.* (1984) examined ways to reduce the effects of rapid imbibition and high leakage of electrolytes in low vigour seed lots of soybean. They found that by reducing the rate of water uptake in aged soybean seeds electrolyte leakage was reduced and germination improved.

Pollock *et al.* (1969) tested the germination of 12 commercial seed lots of garden beans. It was reported that transverse cotyledonary cracks in crack sensitive varieties increased when seeds were imbibed at a lower initial seed moisture content.

Peske (1983) planted seeds of soybean cultivar Davis and Bragg in silty clay loam and sandy loam soil. Emergence was reduced under both low or high soil moisture conditions. Soil moisture contents of between 13 to 16% and 8.5 to 13% were optimum in clay loam and sandy loam soils respectively. If seed moisture content was increased in soils where water uptake was still possible, but germination inhibited, the seeds deteriorated and lost the capacity to germinate and emerge. Water logging reduced germination due to anoxia and injury from rapid imbibition.

Experiments conducted by Tyagi & Tripathi (1983) and Bharati *et al.* (1983) showed that a soil temperature of between 24°C to 32°C, was optimum for soybean germination. However, in one of the experiment a constant temperature of 40°C, appeared to be near maximum for germination (Hatfield & Egli, 1974). A daily

exposure of soybean for 4 h to a germination temperature of 38°C to 40°C, reduced seedling elongation (Emerson & Minor, 1982).

Low temperature and high osmotic concentration reduced and delayed water uptake in lima beans (Pollock & Toole, 1966), however, germination and emergence of soybean at temperatures of 10°C or below were very slow (Matthews & Hayes, 1982). Maximum vegetative growth in soybeans occurred at 30°C (Brown, 1960).

Differences in the response of cultivars to temperature were illustrated by Wallace (1988) who germinated seeds of soybean cultivar Bragg and Cobb in sand at temperature ranging from 25°C to 40°C. Emergence decreased when temperature increased above 37°C, with virtually no emergence at 40°C. Emergence of 12 other cultivars at 38°C, ranged from 25 to 95%. Foster and Coker-338 were more sensitive to high temperature than other cultivars.

Mortensen *et al.* (1986) reported that difference in response of cultivars has also been illustrated in artificially aged and control seeds of 2 cold tolerant and 2 cold susceptible soybean genotypes. Seeds were exposed to 10°C or 25°C, and 0.2, 2 or 20% oxygen during imbibition. Reduced oxygen availability decreased seed and seedling performance in high quality (unaged) seeds more than in low quality (aged) seeds and more at higher temperature than at lower temperature. Moreover, a temporary cessation of germination occurred at 0.2% oxygen.

Rajput & Sastry (1985) studied the effects of depth of planting on seedling emergence of three soybean cultivars. A decrease in seedling emergence was observed when seeding depth increased from 2.5 to 5 cm and 7.5 cm. The ideal seeding depth was 2.5 to 3 cm.

2.7.2. Soil fertility and seedbed conditions

Pakistan can rightly be called as the museum of soils (Saleem *et al.*, 1986). The

variation is largely due to differences in geological age, parent material and climate. Extensive areas that are suited to large scale production of soybeans are quite old. Most of the soils in Pakistan have developed from deeply weathered materials.

Agricultural fields in Pakistan are generally well drained (aerated) and possess adequate mineral reserves for supplying nutrients to plants, but very low in nitrogen, phosphorous and frequently potassium. However, these deficiencies can be supplied through artificial fertilisation (Saleem *et al.*, 1986) However, if properly inoculated with the right rhizobia soybean crop can produce its own nitrogen, however, small amount of nitrogen (20 kg per hectare) is needed as a starter (Roughley, 1980).

In developed countries, most farms are large, and land preparation and planting are done solely by machines. On the other hand, in the developing countries, farm sizes are small, and most of the farm practices are done by farmers using small hand tools (Petcharat, 1982).

Land preparation makes the soil suitable for planting, encourages the seed to germinate quickly, and helps the roots of the young seedling to take up moisture and nutrients from the soil. Good land preparation leads to high yields when appropriate production technology is used. Petcharat (1982) reported that soybean does produce optimum crop stand and high yields on light sandy loam to medium soils with good drainage. A good conventionally prepared seedbed for soybean should be free from weeds, well aerated and should have proper tilth and moisture for good seed germination and emergence (Hinson & Hartwig, 1982).

Leudders & Burris (1979) have reported higher than average germination of soybean in sand compared to rolled paper towels, however, percentages of broken seed coats and abnormal seedlings were negatively correlated with germination and positively correlated with each other.

After a greenhouse experiment by Helsel *et al.* (1985) it was suggested that

sowing of soybean in cold wet conditions can limit germination, emergence and subsequent growth. Therefore, greenhouse and field trials were conducted to evaluate chemical and mechanical seed-bed treatments to reduce the negative effects of these stresses. Narrow black bands of black paint or cinders and ridging of soil over seed planted on the soil surface increased temperature and emergence speed and resulted in early growth.

Rathore *et al.* (1983) reported that seed-beds composed of fine aggregates gave the highest and most rapid seedling emergence compared with coarse aggregate seed-beds. There were larger mechanical hindrances to emerging seedlings on coarse seed-beds due to greater elongation required to grow around the aggregates. This caused the hypocotyl to be kinked. The results suggested that the hazards of surface crusting can be reduced by stratification of the seed-bed with finer aggregates in the seed zone and coarser aggregates near the surface.

2.8. ACCELERATED AGEING TEST

In accelerated ageing test the seeds are rapidly aged by exposure to high temperatures and high relative humidity; e.g. for soybean a favourable treatment may be 41°C and 100% relative humidity. After short periods (2 to 4 days) the seeds are tested by the standard germination test. The accelerated ageing test was originally developed to estimate longevity in storage (Delouche & Baskin, 1973). However, it has also been used to determine seed vigour during seed production in wheat (Rasyad *et al.*, 1990) and attempted to predict field emergence in soybean (Tekrony & Egli, 1977).

Deterioration of soybean seed during storage is more closely associated with the initial vigour level of the seed than with germination. The accelerated ageing (AA) test a common measure of seed vigour, has been shown to correlate well with the changes in germination and vigour during storage (Delouche & Baskin, 1973; Tomes *et al.*, 1988). Unfortunately, these comparisons can only relate initial to final quality

and do not account for temperature and seed moisture variations which occur between storage environments. Large variations have been recorded in the results of accelerated ageing tests (McDonald, 1977), but Spain (1982) suggested that these variations can be reduced by (a) evaluating seed on weight rather than on a seed number basis; (b) controlling temperature and relative humidity precisely; and (c) measuring the initial and final seed moisture content.

The rapid deterioration of seed lots subjected to both high temperature and high relative humidity under laboratory conditions has been of significance in the field of seed ageing. Following the suggestions of Delouche & Baskin (1973) accelerated ageing of seed lots over several days of exposure to 40°C and saturating humidity has been recognised as a good predictor of storability; those seeds that deteriorate rapidly under conditions of accelerated ageing also tend to perform poorly in long-term open storage. The Association of Official Seed Analysts has recognised accelerated ageing as a useful vigour test for some species, including soybean (AOSA, 1983).

Accelerated ageing is useful in determining the storage potential of seed lots, because high initial germination of a particular seed lot is not an assurance that the lot will keep well in storage. Seed lots deteriorate rapidly under high temperatures and humidity. High temperature (41°C) and a relative humidity close to 100% is used on a sample of any particular seed lot to obtain a preview of what the germination of that lot may be if it is put into storage for six months, one year or longer. Accelerated ageing saves time by subjecting the seeds to rapid deterioration that would require months or even years to occur under natural storage conditions.

Tomes *et al.* (1988) used the inner chamber procedure (controlled relative humidity and temperature) and evaluated the influence of the ageing chamber and several test variables on germination of soybean after accelerated ageing. It was observed that ageing time, temperature and seed moisture content interacted during ageing to reduce seed germination. Temperature had the greatest effect on

germination and 41°C was a threshold of sensitivity for soybean seeds. An ageing time of 72 h provided excellent separation in vigour across seed lots. Some seed deterioration was detected after 48 h and excessive deterioration occurred after 96 h. Relative humidity in the inner chamber reached 90% after 24 h and gradually increased to 95% by 72 h. Final seed moisture content after ageing was 31 to 34% and was one of the most repeatable aspects of the test. They further stated that initial seed moisture had little effect on the final seed moisture content; but both initial and final seed moisture contents should be monitored during ageing.

2.9. CONTROLLED DETERIORATION

Like the accelerated ageing test, controlled deterioration is based on the principles of seed ageing, however, here there is a closer control of seed moisture content and temperature. Seeds are deteriorated at known moisture contents for 1 day at 45°C. After 1 day of accelerated ageing deterioration at 45°C, germination of the seed lots reflects the vigour of the seed, thus good germination indicates high vigour (Delouche & Baskin 1973).

Although laboratory germination tests give a very good assessment of the ability of seeds to produce a seedling, often in the field situation, seeds fail to emerge. This problem is greatest in poor weather conditions. Thus in similar conditions, some seed lots will emerge well and others badly, despite similar laboratory germination. Therefore, the controlled deterioration test predicts the relative performance of seed lots in the field, picking out low vigour lots (poor emergers) and high vigour lots (good emergers).

Differences in the vigour of highly germinable seed lots can also lead to problems in storage, since the differences in vigour mean differences in the storage potential of the seed (Lassim and Delouche, 1981). Thus of two seed lots with high initial germination as they go into store, the low vigour lot will show a more rapid decline in

its germination during a period of storage than high vigour seed lot.

2.10. PREDICTION OF FIELD PERFORMANCE OF SEED LOTS

Generally, soybean seeds are stored from harvest until the next planting season, but some seed producers show interest in longer storage periods. However, measure of soybean seed quality (germination and vigour) at harvest does not provide enough information to estimate changes in seed quality during storage.

It has already been reported earlier in this review that the major problem that has hindered a rather more accurate prediction of the life of a seed lot is the variation in viability period from seed to seed. Such variation has suggested to many workers that seeds are unpredictable and consequently to perceive laws of behaviour in such uncertain individuals would be a waste of time. However, Roberts (1973a) concluded that although the behaviour of any individual seed in a population is difficult to predict; the behaviour of populations of seeds can often be defined very accurately. Various seed deterioration prediction models that include the effect of seed moisture and storage temperature have been developed (Hukill, 1963; Roberts, 1973a; Roberts, 1984). These models have been revised and expanded to include many crop species, including soybean (Ellis *et al.*, 1982).

Soybean seeds deteriorate rapidly during storage (Chauhan, 1985). Factors which influence seed deterioration in storage is seed moisture, temperature and the initial quality of the seed (Roberts, 1984). It has been observed that when soybean seeds are stored at high temperature or high seed moisture levels, germination will decline more rapidly compared to seed stored in cooler and drier conditions. Taking into consideration the relationship of seed moisture, storage temperature and a few constants it is possible to predict the storage life of soybean under a wide range of storage environments. This has been illustrated in Table 2.1. For details refer to Ellis

& Roberts (1980a).

Table 2.1. Suggested combination of seed moisture content (% wet basis) and storage temperature at which soybean viability will decline from 95 to 85% during storage.

Storage months	10°C	15°C	20°C	25°C	30°C
9	11.5	10.5	9.0	6.5	6.5
18	10.0	8.5	7.5	5.5	5.5
36	8.0	7.5	6.5	4.5	4.5

Tekrony *et al.* (1993) tested the accuracy of Ellis & Roberts (1980a) seed deterioration predictive model for seed germination during warehouse storage of soybean. It was reported that the model offers potential for predicting the germination of soybean seeds in normal warehouse storage, if warehouse temperature and seed moisture are known. The model accurately predicted germination (± 10%) for 15 of the 17 seed lots for 4 and 16 months storage. The two seed lots with large differences between measured and predicted germination were those with higher levels of mechanical injury.

2.11. AIMS AND OBJECTIVES

Some of the serious problems hindering expansion of soybean in the plains of Pakistan are summarised in chapter 1. Consideration of the available literature indicated that the solution to these problems is to provide farmers with seeds of good storability and high germinability. It is also important to enhance actual germination in the field. Enhanced germination and early emergence may enable the seedlings to penetrate their roots deep into the soil and to emerge out of the soil before loss of soil moisture or crusting of the soil. This thesis intends to:

(i) Evaluate the effect of seed quality and storage environment on germinability of soybean seeds. To study the relationship between laboratory germination and tetrazolium topographic staining tests and field emergence.

(ii) Evaluate the effect that slow imbibition and osmotic stress have on germinability and speed of germination in unaged and aged seeds.

(iii) Investigate various soybean cultivars for storage potential and resistance to imbibition damage and to identify seed characteristics responsible for these traits.

CHAPTER 3

MATERIALS AND METHODS

3.1. INTRODUCTION

General materials and methods are described in this chapter whereas specific methods relating to each experiment are described in successive chapters.

The experiments were conducted in the laboratories and Plant Growth Unit (PGU) of the Department of Crop Science and Technology, The Scottish Agricultural College, Edinburgh centre of study. The field emergence test in case of experiment 4.1 was performed at the Agricultural Research Station Mingora, North West Frontier Province, Pakistan.

3.2. SEED SOURCE

Seeds were supplied by the Department of Agronomy, College of Agriculture, University of Maryland, USA, and North West Frontier Province Agricultural University Research Station Mingora, Pakistan. Seeds of two Indian cultivars were obtained from Dr. M. Turner, and seeds of two French cultivars were supplied by Dr. P. Koole, here at the School of Agriculture (Table 3.1). The initial seed moisture content, germination capacity, hundred seed weight, seed colour, seed size and hilum colour was recorded.

The initial germination capacity of the seeds was determined in rolled paper towels at 25°C, using two replications of 25 seeds. Hundred seed weight was based on average of three seed samples of 100 seeds for each cultivar. For each cultivar the

seed was described as either small (S), medium (M) or large (L) at a uniform 10% seed moisture. Small seeds weighed between 10 to 15 g, medium seeds 15 to 18 g and large seeds > 18 g. Details of the seed characteristics of each cultivar are listed in table 3.1.

3.3. DETERMINATION OF SEED MOISTURE CONTENT

Seed moisture content was calculated by using two seed samples, between 10 and 10.5 g each (W1). Seeds were placed in glass beakers and dried in an oven for 16 h at a constant temperature (105°C). The seeds were then broken down and dried until a constant weight was obtained. The weight of the seed samples after drying was recorded (W2). The seed moisture percentage was calculated on fresh weight basis (the product of loss in weight of the seed sample x 100, divided by initial weight of the seed sample). The mean of the moisture percentage of the two seed samples was calculated as the initial moisture content of the seeds.

3.4. ACCELERATED AGEING TECHNIQUES

Low vigour seeds samples were produced by enhancing the process of seed deterioration using one of the following two methods:

3.4.1. Method 1

In early experiments seed was aged in an accelerated ageing chamber (600G3, THTL, FISONS Scientific) adjusted to 41°C (80-100% relative humidity). The seeds were placed in plastic plates covered with a double layer of muslin to protect against condensation. The required high relative humidity (80 to 100%) at high temperature (41°C) proved to be beyond the capacity of the ageing chamber and as such this method of accelerated ageing was disappointing and subsequently abandoned.

Table 3.1. Characteristics of soybean cultivars used in this study.

Cultivar	Seed coat colour	Hilum colour	Initial moisture content	100 seed wt (10% mc)	Seed size
Source: Pakistan					
Cumberland	Y	BL	8.17	20.35	L
Epps	Y	BL	8.68	20.23	L
Pixie	Y	BL	9.39	20.64	L
Swat-84	Y	BL	9.38	19.75	L
Weber	Y	BL	8.71	11.59	S
Woodworth	Y	BR	9.27	17.95	M
79-W-220	Y	BL	9.40	16.00	M
80-B-4007	Y	BR	9.16	15.43	M
Source: USA					
Conrad	Y	BL	07.11	16.24	M
Emerald	G	BL	07.65	26.52	L
Essex	Y	BR	08.77	14.52	S
Kent	Y	BL	11.64	16.38	M
Linford	Y	BL	10.79	16.25	M
Manokin	Y	BR	10.46	14.24	S
Morgan	Y	BL	13.23	18.08	L
Ripley	Y	BR	14.02	13.94	S
Stafford	Y	BR	09.58	13.41	S
Stonewell	Y	BL	9.30	18.47	L
York	Y	BR	11.44	18.85	L
Source: India					
Kalitur	BL	W	11.32	14.07	S
Pb-1	Y	BR	8.43	11.27	S
Source: France					
Gemma	Y	BR	11.07	22.41	L
Opal	Y	BR	10.93	19.39	L

Seed or hilum Colours: BL=Black BR=Brown Y=Yellow
G=Green W=Whitish

Seed Size/100 seed wt: S=Very small/10-15 g M= Small/15-18 g M= Medium/>18 g

3.4.2. Method 2

A sealed desiccator containing distilled water was placed in a water bath running at 41°C. Seeds were spread in a single layer on muslin cloth on a metallic wire mesh. The wire mesh was placed inside the desiccator such that the seeds were situated about 3 cm to 4 cm above the water. The desiccator was sealed, and thus 95 to 100% relative humidity could be maintained. Seeds were protected against condensation by wrapping the cover of the desiccator in a double layer of muslin. This method of accelerated ageing was reliable and guaranteed uniform and desired accelerated ageing conditions for the required time.

3.5. SEED MOISTURE ADJUSTMENT

The required number of seeds of known moisture content were weighed to two decimal places. If a higher moisture content was required then seeds were incubated in a sealed desiccator, containing distilled water at 20°C for 2-5 days. Conversely if a lower moisture content was required then seeds were placed in a sealed desiccator containing blue silica gel and incubated at 20°C to 25°C for 2-5 days.

The seeds were weighed after intervals of 2-12 hours depending upon the difference between the calculated and desired moisture content unless the desired weight of the seeds to be at the desired moisture content was attained. In some cases the seeds gained or lost more weight than required, and were re-adjusted in the desiccator containing blue silica gel or distilled water as needed. The expected weight of the seed samples at the desired moisture percentage was calculated using the following formula:

$$DW = 100 - OSM / 100 - DSM \times IW$$

Where DW, is the desired weight of the seed sample. OSM is the original seed moisture content of the seed sample. DSM is the desired seed moisture content, and

IW is the initial weight of the seed sample. After moisture adjustment, the seeds were sealed in polythene bags and were then placed in a cold room (4°C) for at least 2 days to equilibrate moisture between the seeds.

3.6. IMBIBITION IN POLYETHYLENE GLYCOL (PEG)

The method of preparation of PEG solutions of various concentrations was such that to make a 25% solution, 25 g of PEG (Mol. Wt. 8000) was dissolved in 75 ml distilled water. An electro-magnetic stirrer was used to dissolve the solution completely. Pre-planting incubation was performed by using one of the following methods.

3.6.1. Method 1

Samples of seeds each imbibed in beakers containing solutions at various PEG concentrations (10%, 25%, and 50%). Imbibition was achieved at 20°C, in Sanyo Gallenkamp Cool Temperature Cycling Incubator, INF-631 (Table 3.2).

3.6.2. Method 2

Seeds were imbibed between rolled paper towels applied with distilled water or PEG at 20°C, in Sanyo Gallenkamp Cool Temperature Cycling incubator (INF-631). Specific methods relating to each experiment show the concentrations used (Chapter 5).

3.7. SEED TREATMENT

During these experiments seeds were exposed to conditions conducive to fungal attack. Therefore, before germination test seeds were either dipped in 1% sodium hypochlorite for 5 seconds and rinsed 3 to 4 times with distilled water or treated with

Dithane M-45 @ 1.5 g per Kg of seed. All incubators and the growth room were sprayed with 70% ethanol before germination test (Table 3.2).

3.8. GERMINATION TESTS

Seed germination was studied using one of the following methods:

3.8.1. Method 1

One and a half metre long single layers of 19 cm wide non toxic Kimberly Clark (Table 3.2) paper towels were uniformly moistened by soaking them for ten minutes in distilled water followed by a squeeze between a plastic foot ruler and the surface of laboratory table Seeds were placed 4 to 6 cm apart in a single row on a double layer of moistened paper towel. The seeds were covered with another single layer of paper towel. The paper towels were rolled-up 8 to 12 times and enclosed in polythene bags. The polythene bags were placed upright in Sanyo Gallenkamp Cool Temperature Cycling incubator in dark (Table 3.2). Temperature varies as shown in specific methods of each experiment in successive chapters.

3.8.2. Method 2

For germination in compost or sand, the seeds were planted 2.5 to 3 cm deep in plastic trays or pots in a growth room (25°C; 12 h/d photoperiod) or inside a greenhouse respectively (between 12 to 21°C). Full appearance of both the cotyledons on the surface was regarded as completion of emergence and evidence of capacity to establish.

3.9. CONDUCTIVITY TEST

Conductivity test associates the extent of electrolyte leakage out of imbibing seeds to their quality (potential for field emergence).

Table 3.2. List of equipment and chemicals used.

Name	Grade	Supplier	Product code
Equipment			
Ageing chamber	600G3/THTL	FISONS SCIENTIFIC APPARATUS	0600/08
Blotting paper	Seed test grade 90 mm	Whatman Scientific	2181090
Compost	Sphagnum Moss Peat	L & P Ltd.	PBL 020044
Conductivity meter	Digital 10-2 to 10-6	WPA	CND-400
Desiccator	279 mm x 330 mm	COLE-PARMER	G-06520
Incubator	Cool temperature cycling	Sanyo Gallenkamp	INF-631
Paper towel	19cm x 76.5	Kimberly-Clark	7385
Petri dishes	Triple Vent 90 mm	Sterilin or Philip Harris	P35-525
Sand	Washed 3 mm down	Silvaperl	5 010672 149254
Test tubes	200 x 24 mm	SAMCO Scientific	G-001
Water bath	ZA	Grand Instrum. Camb. England	038710058
Chemicals			
2,3,5-triphenyl tetrazolium chloride	Anhydrous Mol. Wt: 334.8	SIGMA	T-8877
Dithane 945	Mancozeb	ROHIM & HASS UK Ltd.	No: 2085
Ethanol (70%)	General Purpose Reagent	B.D.H	28304
K_2HPO_4	FW-105	M & B Lab. Chemicals	Lot-66570
Na_2HPO_4	FW-109	SIGMA Lab. Chemicals	S-5012
Polyethylene glycol	Av. Mol. Wt: 8000	SIGMA	P-2139
Sodium hypochlorite	10 to 14% available Chlorine	Deosan	06055 AD4
Distilled water	Obtained at the Dept. of CST, Scottish Agricultural College, Edinburgh		
High purity water	Obtained at the Dept. of Soil Science, Scottish Agricultural College, Edinburgh		

The degree of solute leakage measured by leachate conductivity from the seeds was monitored by soaking 25 weighed seeds per replication in 35 ml distilled water for the desired time (varies with experiment) at the desired temperature (25°C or 35°C, or both depending upon experiment). Details are given in specific methods in successive chapters. In experiment 6.3, high purity water was used. Before soaking, all seed material was placed in an incubator for at least 4 to 5 h to equilibrate to the specified temperature. The conductivity of blank distilled water was measured with the help of a conductivity meter. A reading of 0.0 was ensured with the dry cell using the lead compensation and cell correction controls. The electrode of the conductivity meter was rinsed in distilled water and dried before transferring between solutions. The conductivity of the soak solution was expressed per gram of seeds for each replication (μSg^{-1}). The mean of the two replications indicated the degree of solute leakage from the seeds.

3.10. OBSERVATIONS

Seed germination results were classified into the number of normal or abnormal seedlings and number of ungerminated seeds according to the International Seed Testing Association (ISTA) Rules (1985), simplified as shown in table 3.3.

Table 3.3. Differentiation of normal seedlings, abnormal seedlings and ungerminated seeds.

Normal seedlings
1. A well developed root system including primary root with root hairs. 2. A well developed shoot including straight hypocotyl and two cotyledons. 3. Primary roots with limited damage, but well developed secondary roots. 4. Hypocotyl with limited damage. 5. Cotyledons with limited damage. 6. Only one normal cotyledon. 7. Only one normal primary leaf.
Abnormal seedlings
1. Primary root stunted, retarded, broken, missing, trapped in the seed coat, glassy, decayed due to primary infection. 2. Hypocotyl short or swollen, deeply cracked, broken, missing, tightly twisted, bent over, decayed due to infection. 3. Cotyledons swollen, curled, deformed, broken, separate, missing, necrotic, and decayed as a result of infection. 4. The primary leaves deformed, damaged, missing, decayed as a result of infection. 5. The seedling as a whole deformed, twisted, discoloured, spindly, glassy or decayed as a result of infection
Ungerminated seeds
1. Seeds with no part of the seedling being produced.

The germination process was complete when the radicle of the seedling was 2 cm long, together with potential evidence of shoot growth.

Data recorded on shoot length, hypocotyl length, seedling fresh weight, shoot fresh weight and seedling dry weight were recorded on 5 randomly selected seedlings. Dry weight was obtained after the shoots were allowed to dry overnight in an oven at 85°C. Shoot length and hypocotyl lengths were the mean of 5 random data.

3.11. STATISTICAL APPROACH

Germination tests in rolled paper towels were arranged in incubators according to a completely randomised design. However, field emergence conducted at Agricultural Research Station, Mingora, Pakistan, and germination tests in sand or compost in the growth room or green house were arranged according to a randomised complete block design (RCB). The data were analysed with the help of a statistical package (Minitab Release 7.1 VAX/VMS version) available at the Scottish Agricultural College Edinburgh. A least significant difference value (LSD at 5% level of confidence) was calculated to compare the means. LSD value is shown in the table of means or as error bar in the bar charts of mean values. The LSD was calculated according to the following formula:

$$LSD = t_{0.05} \sqrt{\frac{2 \times EMS \text{ (error mean square)}}{\text{No. of replications}}}$$

The ANOVA significance tests were performed on all data where appropriate. A probability value of less than or equal to 0.05 indicated a significant effect.

CHAPTER 4

RELATIONSHIP OF LABORATORY TESTS TO FIELD EMERGENCE AND EVALUATION OF SOME IMPORTANT ENVIRONMENTAL FACTORS AFFECTING SEED STORAGE LIFE AND GERMINATION

4.1. INTRODUCTION

The germinability of a seed lot is measured to assess its suitability for field planting value (Mackay, 1972). Researchers believe that dependence on a single viability test is not sufficient. Therefore, different tests should be used especially, if seeds are to be planted in adverse soil conditions (Tekrony & Egli, 1977).

Tetrazolium topographical staining and the standard germination test are widely used as predictors of the field emergence capacity of seeds (Yaklich *et al.*, 1979; Yaklich & Kulik, 1979; Overaa, 1982). In many tropical and subtropical countries, however, seeds are stored in non ideal conditions characterised by a combination of high temperature and high relative humidity. These conditions contribute to rapid seed deterioration during storage (Cooper, 1959; Naudi *et al.*, 1982; Arulnandhy & Senanayake, 1988). Overestimation of the field emergence capacity (under laboratory conditions) of seeds with low potential vigour may lead to even lower establishment in the field.

In addition, seed vigour is determined by the conditions and length of storage (Delouche *et al.*, 1973). Environmental factors during storage that most markedly influence maintenance of seed vigour and viability are temperature and seed moisture content (Ellis, 1982).

In hot tropical and subtropical regions it is the intrinsic relative humidity that determines the moisture content of the seeds. High relative humidity encourages moisture re-absorption and rapid seed deterioration especially if storage temperature is high (Amable & Obendorf, 1986). For example, soybean seeds at a moisture content of 14.7% (equilibrium seed moisture content at approximately 80% relative humidity) lost more than half of their initial viability in 12 weeks when kept at 30°C (Dorworth & Christensen, 1968).

On the other hand postharvest handling in tropical and subtropical countries does not guarantee that seeds will be dried adequately (<10% moisture). This makes the seeds unsuitable for sealed storage (Delouche *et al.*, 1973; Gregg, 1982). Soybean seeds are particularly vulnerable to irreparable damage under unfavourable storage conditions (Chauhan, 1985).

It is not possible to stop the process of seed deterioration, but appropriate storage conditions can retard seed deterioration and enable the seeds to maintain high seed vigour and viability for many years (Harrington & Douglas, 1970; Delouche, 1982). Results of several experiments have shown that either low temperature or low seed moisture content is essential for the maintenance of good seed viability, however, in some cases both may be required (Dorworth & Christensen, 1968).

Another reason for poor emergence and an unsatisfactory crop stand of soybean is that farmers in the tropical and subtropical countries do not know that seeds with a low moisture content, if exposed to rapid water take up suffer from severe injury especially during the initial phase of imbibition (Parrish & Leopold, 1977).

In an experiment by Hobbs & Obendorf (1972) the low initial moisture content of soybean seed reduced germinability and this was attributed to high solute leakage. However, firm conclusions have not been drawn on the effect that initial seed moisture content, germination temperature and their interaction may have on

germinability of soybean seeds.

Soybean seeds stored at a low moisture level (8% or less) will maintain high vigour (Delouche, 1974). However, seeds at very low moisture contents (8% or less) are susceptible to soaking injury especially if subject to high soil moisture conditions (Hobbs & Obendorf, 1972; Koslanund & Delouche, 1987).

The problem of seed coat cracking is particularly severe in soybean, especially, in modern cultivars (Lassim & Delouche, 1981). A consideration of the extent of mechanical damage is important before firm conclusions can be drawn. Cracks in the seeds that extend deep into the cotyledons are very hazardous. However, some soybean cultivars are more likely to suffer from minor cracks than others (Singh & Seitia, 1974; Ragus, 1987).

Past research work has shown conflicting results on the negative effects of slightly injured seeds. Toole (1950) was unable to confirm if mechanical injuries could predispose the seed to rapid deterioration compared to uninjured seeds. Other researchers believe that during harvest and postharvest handling soybean seeds are frequently subjected to mechanical injuries (Gupta, 1976) that encourage cracks in the seed coat and thus expose the seeds to rapid quality loss during storage and severe damage during the early phases of imbibition (Sorrells & Pappelis, 1976).

In fact soybean cultivars vary in their susceptibility to soaking injury. The seed coats play an important role in germination success of the seeds because they control the rate of water uptake during germination (McDonald *et al.*, 1988a). A major cause of poor seed vigour in soybean has been identified as the incidence of cracks in the seed coat particularly when the cracks extend deep into the cotyledons, thus allowing rapid water uptake and high solute leakage (Potts *et al.*, 1978).

The above literature convince one to study the relationship between laboratory germination and tetrazolium topographic staining test and field emergence and

evaluate the effect of seed quality and storage environment on germinability of soybean seeds.

4.2. SPECIFIC METHODS

4.2.1. Experiment 4.1

Relationship of laboratory germination, solute leakage and tetrazolium topographic staining to field emergence in ageing soybean (*Glycine max* L. Merrill) seeds.

Seeds of the cultivar Morgan were aged for 2, 4, 6, 8 or 10 days in an ageing chamber (41°C; between 80% and 100% Relative Humidity). An unaged seed sample was used as the control. After ageing all the seeds were treated with Dithane M-45 and were then allowed to dry to a uniform moisture content of 10% at room temperature.

Seeds were germinated between rolled paper towels in incubators at a constant temperature of 25°C (standard germination test temperature), 30°C or 35°C in dark. Four replications of 25 seeds each were used. Data on the number of normal seedlings, abnormal seedlings, ungerminated seeds and seedling fresh weights from each replicate were recorded after 8 days incubation.

For tetrazolium topographic staining four replications of 25 seeds were used. To facilitate the removal of seed coat the seeds were soaked in distilled water at 20°C, for 5 h. The seed coats were carefully removed using a sharp scalpel. The decoated seeds were then allowed to stain for 6 h in a buffered 0.5% 2,3,5-triphenyl tetrazolium chloride salt solution in the dark. The buffer solution was prepared in the following ratio:

4.539 g of potassium hypo phosphate (K_2HPO_4) were dissolved in 500 ml of distilled water (Solution 1).

4.736 g of sodium hypo phosphate (Na_2HPO_4) were dissolved in 500 ml of distilled water (Solution 2).

Two parts of solution (1) were mixed with three parts of solution (2).

Table 4.0. Staining patterns in soybean after tetrazolium treatment
1. Embryo completely stained. 2. Embryo completely stained with dark patches irregularly distributed. 3. Embryo completely stained, but lightly. 4. Embryo stained except for unstained small patches on the periphery of the cotyledons. 5. Embryo stained except for irregular unstained patches on the cotyledons occupying nearly half of the area opposite to the radical. 6. Embryo showing irregular unstained patches on the cotyledons affecting more than half of their area and radical unstained less than half its length towards the tip. 7. Embryo showing an unstained necrotic patch (more or less large) or two small patches on the cotyledons occupying more than half their total area. 8. Embryo unstained for occasional small stained patches here and there. 9. Embryo and cotyledons with only margins or surface, stained or nearly 50% unstained. 10. Embryo completely unstained or radical or cotyledonary end unstained.

Stained seeds were then categorised as potential normal, potential abnormal and incapable of germination using a modification of the procedure followed by Pasha & Das (1982) who classified stained seeds into ten categories according to their staining pattern. Those categories were re-defined such that seeds falling in category 1 to 3 were regarded as capable of producing a normal seedling, those falling in category 4 and 5 were expected to be capable of producing only abnormal seedlings and those falling in category 6 to 10 were regarded as non viable or seeds incapable of germination under favourable field conditions (Table 4.0).

In most seeds the failure of the tip of the radical to stain was accompanied by major unstained patches on the cotyledons. However, some seeds were regarded as ungerminated even if more than half of the tip of the radical alone remained unstained.

To study the effect of temperature and ageing on the conductivity of seed exudates two weighed replications of 25 seeds each were soaked for 5 h in beakers containing 35 ml distilled water at 25°C or 35°C. The soak water was transferred to a measuring cylinder and a conductivity reading was recorded (μSg^{-1}).

A field emergence test was conducted on a well prepared seedbed at the Agricultural Research Station, Mingora, Pakistan. Four replications of 25 seeds per treatment were planted, each in 1m long rows 45 cm apart according to a randomised

complete block design. Data were recorded on normal seedlings and abnormal seedlings. Abnormal seedlings mostly included those that failed to emerge or showed potential for normal growth after emergence. The percentage of normal and abnormal seedlings was added up and subtracted from 100 to get the percentage of ungerminated seeds.

The standard germination test conducted in rolled paper towels at 25°C, was compared with tetrazolium topographic staining and field emergence test. In addition, comparison between germinability at three incubation temperatures (25°C, 30°C or 35°C) was also made. The conductivity measurement of the seed samples at 25°C or 35°C, monitored the relationship of solute leakage to field emergence (μSg^{-1}). Details are given in chapter 3.

4.2.2. Experiment 4.2

Action and interaction of seed moisture, temperature and time during sealed storage.

Seeds of the cultivar Morgan were adjusted to 8%, 12% or 16% seed moisture content. The seeds were sealed in test tubes and exposed to artificial storage for 7, 14 and 21 days at 35°C or 40°C (controlled deterioration). A piece of cotton wrapped in aluminium foil was inserted in the test tubes to allow a minimum of air space.

The test tubes were kept submerged upright in stands in two water baths running at the required temperature. After treatment seed samples were sealed in polythene bags and stored in a cold room (5°C) until all the various seed treatments were completed.

To avoid any effect of initial seed moisture content at the onset of germination the seeds at 8% or 16% moistures content were also adjusted to 12% moisture content before the germination test. All the seeds were treated for 5 seconds in 1% sodium

hypochlorite before the germination test.

A germination test was conducted in rolled paper towels in an incubator at 25°C in dark. Four replications of 25 seeds each were used. Data were recorded on the number of normal seedlings, number of abnormal seedlings and number of ungerminated seeds after 8 days. Details are given in chapter 3.

4.2.3. Experiment 4.3

Temperature, initial seed moisture and their interaction in unaged and aged seeds during germination.

Seeds were subjected to accelerated ageing for 3 days in a sealed desiccator placed in a water bath at 41°C; between 95% and 100% relative humidity. The seeds attained a moisture content of 31% during the accelerated ageing process. An unaged seed sample was the control. Both the aged and unaged seeds were subsequently adjusted to 6%, 12% or 16% moisture content.

Seeds were treated for 5 seconds in 1% sodium hypochlorite and then germinated between rolled paper towels in incubators at 17°C, 25°C or 35°C in dark. Data were recorded on the number of normal seedlings, number of abnormal seedlings and number of ungerminated seeds. The number of hours taken to 50% germination was also recorded.

Two replications of 25 seeds each were soaked in 35 ml distilled water at 25°C or 35°C for 30 minutes. The conductivity of soak water was then measured. Seeds at 12% initial moisture content were used as a control seed sample.

The germination test at 25°C was used as a check on germination at 17°C or 35°C. The time taken to 50% germination at 25°C was compared to the time taken at 17°C or 35°C. The conductivity recorded at 25°C was compared to that at 35°C. Details are given in chapter 3.

4.2.4. Experiment 4.4

Effect of seed <u>etching</u> (cut on both cotyledons) on germinability of unaged and aged seed samples of three soybean cultivars.

Healthy looking seeds from cultivars with small seeds Essex and Pb-1 and cultivar Gemma with large seeds were subjected to accelerated ageing for 4 days in a sealed desiccator placed in a water bath (41°C; between 95% and 100% relative humidity). An unaged seed sample of each cultivar adjusted to about 20% seed moisture content acted as the control.

Seeds at 20% moisture were easily scarified with a sharp scalpel. Scarified seed samples were obtained by producing a 1.5 mm to 2 mm long scratch on both the cotyledons of apparently undamaged seeds. Separate batch of un-scratched seeds was used as control. All seeds were allowed to dry back at room temperature for 3 days.

Seeds were treated for 5 seconds in 1% sodium hypochlorite and then a germination test was conducted in sterilised sand in a growth room (25°C; 12 h/d photoperiod) using 4 replications 25 seeds each. Data were recorded on the number of normal and number of abnormal seedlings after 12 days. An estimate of the number of ungerminated seeds was also made.

The sand was uniformly moistened during the germination test. To monitor the degree of solute leakage two replications of 25 seeds each were soaked for 4 h in 35 ml distilled water and a conductivity measurement (μSg^{-1}) was recorded. Details are given in chapter 3.

4.3. RESULTS

4.3.1. Experiment 4.1

Relationship of laboratory germination, solute leakage and tetrazolium topographic staining to field emergence in ageing soybean (*Glycine max* L. Merrill) seeds.

Seed moisture content increased from 8.5% to 16.9% after two days of accelerated ageing and reached 23.5% after 6 days of accelerated ageing with no further increase thereafter (Table 4.1). As mentioned in chapter 3, 95% to 100% relative humidity at 41°C, proved to be beyond the capacity of the accelerated ageing chamber and as such this method of ageing was disappointing and subsequently abandoned. The relative humidity inside the accelerated ageing chamber fluctuated and so did the moisture content of the seeds. However, successive seed lots showed a useful drop in germinability that was ideal for this type of investigation.

The combined means of the percentage of normal and abnormal germination showed that 86% of the seeds were viable and not killed by 6 days of accelerated ageing, however, after 8 days of accelerated ageing about half of the seeds lost the ability to germinate (Figure 4.1a-c). Ageing seeds showed even poorer germinability when germination temperature was high (35°C). This indicate that loss of germinability due to ageing was a reliable criterion of loss in the ability to germinate at higher temperature (35°C). Poor germinability due to ageing or due to high germination temperature was associated with high solute leakage (Table 4.1). The observed inverse relationship of ageing with germinability and direct relationship with solute leakage can be attributed to the loss of membrane integrity.

4.3.1.1. Relationship between laboratory tests and field emergence

The number of normal seedlings obtained under laboratory conditions in rolled paper

towels (25°C, 30°C, 35°C) and those predicted in the tetrazolium test had a significant correlation with field emergence ($r = 0.9$). However, the reliability of laboratory tests to predict field emergence decreased with accelerated ageing (Figure 4.1a-c). This was, however, attributable to the poor laboratory germination and higher solute leakage from poorly emerging seed lots (Table 4.1).

Field emergence was negatively associated with solute leakage measured by leachate conductivity and also with shoot fresh weight obtained in rolled paper towels ($r = 0.8$). Solute leakage measured by leachate conductivity, however, had a positive association with abnormal seedlings and percentage of ungerminated seeds both under laboratory and field conditions (Figure 4.1a-c; Table 4.1)

The prediction made on tetrazolium staining test and laboratory germination revealed that accelerated ageing caused a more rapid reduction in the number of normal seedlings when germination occurred under field conditions (Figure 4.1a).

The association between laboratory tests also weakened as ageing continued. For example, in case of seed lot subject to 6 days accelerated ageing, the tetrazolium staining test over predicted laboratory germination in rolled paper towels (Figure 4.1a-c).

In the case of control seeds and seeds subject to 2 and 4 days accelerated ageing laboratory tests were a very accurate estimate of the number of normal seedlings under field conditions ($P < 0.6$). However, contrary to the prediction made on the basis of tetrazolium topographic staining pattern the seed samples aged for 6, 8 and 10 days produced a lower number of normal seedlings and higher seedling abnormalities together with higher incidence of ungerminated seeds under field conditions (Figure 4.1a-c).

Laboratory germination in rolled paper towels was a good indicator of field emergence in the seed sample aged for 6 days as well as for those aged for 2 and 4

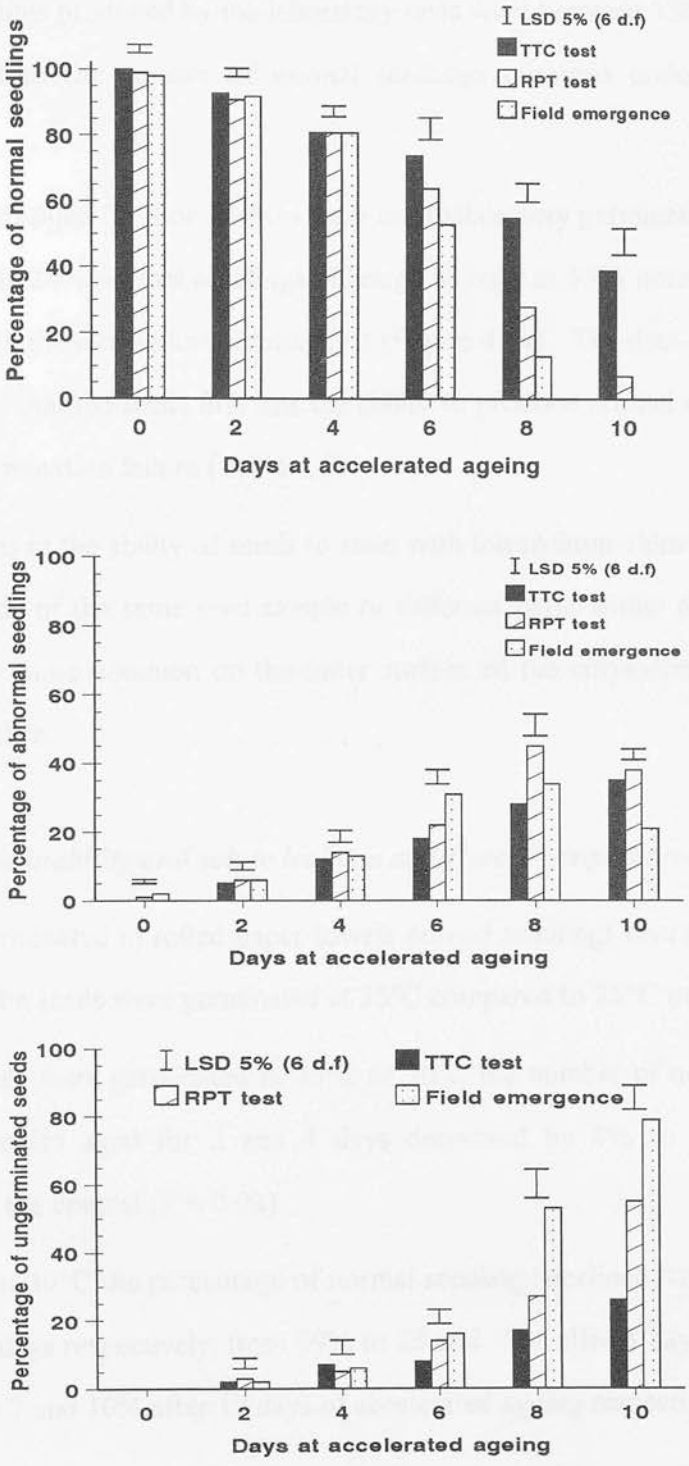


Figure 4.1. Relationship of laboratory tests and tetrazolium topographic staining test to field emergence (a) normal seedlings (b) abnormal seedlings, and (c) ungerminated seeds.

days ($P > 0.2$). However, for seed samples aged for 8 and 10 days the number of normal seedlings produced by the laboratory tests were between 15% and 25% higher as compared to the number of normal seedlings obtained under field conditions ($P < 0.02$).

When seeds aged for 8 or 10 days were used laboratory germination in rolled paper produced only 28% normal seedlings although as high as 55% normal seedlings were predicted with the tetrazolium staining test (Figure 4.1a). The data recorded in all the tests revealed that the seeds first lost the ability to produce normal seedlings followed by a total germination failure (Table 4.1).

Differences in the ability of seeds to stain with tetrazolium chloride existed among different seeds of the same seed sample or different parts within a seed. Unstained patches were more common on the outer surface of the cotyledons as compared to the inner surface.

4.3.1.2. Germinability and solute leakage at different temperatures

For seeds germinated in rolled paper towels normal seedlings decreased with time of ageing or if the seeds were germinated at 35°C compared to 25°C or 30°C ($P < 0.01$).

When seeds were germinated at 25°C or 30°C the number of normal seedlings in the seed samples aged for 2 and 4 days decreased by 8% to 18% respectively compared to the control ($P = 0.02$).

At 25°C or 30°C the percentage of normal seedlings declined from 99% to 64 and 62% after 6 days respectively; from 99% to 28 and 19% after 8 days respectively and from 99% to 7 and 10% after 10 days of accelerated ageing respectively ($P = 0.01$).

At 25°C, 30°C and 35°C, normal seedlings from the control seed lot and the seed lot aged for 2 days declined at a similar rate. However, in case of seed lots aged for 4 and 6 days the decline in the number of normal seedlings at 35°C was 38 and

Table 4.1. Mean data regarding normal seedlings, abnormal seedlings, ungerminated seeds and shoot fresh weight in ageing seeds germinated at three different temperatures in rolled paper towels (mean of four replications). Conductivity at two different temperatures (mean of two replications).

Observation	Accelerated ageing at 41°C (80 to 100% RH)						
	Control	2 days	4 days	6 days	8 days	10 days	LSD at 5% (12 d.f)
Normal seedlings (%)							
25°C	99.0	91.0	81.0	64.0	28.0	7.0	5.4
30°C	99.0	92.0	82.0	62.0	19.0	10.0	5.9
35°C	93.0	88.0	55.0	21.0	0.0	0.0	6.9
LSD at 5% (6 d.f)	4.3	5.4	6.5	6.2	5.8	4.9	
Abnormal seedlings (%)							
25°C	1.0	5.0	13.0	22.0	28.0	36.0	6.3
30°C	0.0	6.0	13.0	21.0	43.0	38.0	5.6
35°C	1.0	9.0	13.0	29.0	23.0	13.0	5.7
LSD at 5% (6 d.f)	*	4.3	2.9	5.4	5.7	6.3	
Ungerminated seeds (%)							
25°C	0.0	4.0	6.0	14.0	44.0	57.0	6.9
30°C	1.0	2.0	5.0	17.0	38.0	52.0	7.1
35°C	6.0	3.0	32.0	50.0	77.0	87.0	6.7
LSD at 5% (6 d.f)	4.3	4.9	6.4	6.7	7.3	6.8	
Shoot fresh weight (grams)							
25°C	6.4	6.7	6.4	4.0	3.8	3.5	1.9
30°C	6.9	7.1	6.8	4.1	3.8	3.6	1.5
35°C	4.0	4.6	4.4	2.2	0.0	0.0	2.7
LSD at 5% (6 d.f)	0.9	1.3	1.7	1.4	0.6	1.3	
Conductivity (μSg^{-1})							
25°C	53.6	57.2	71.2	79.8	100.9	135.4	7.4
35°C	71.7	76.5	86.5	104.1	107.8	154.8	9.9
LSD at 5% (2 d.f)	7.8	6.9	8.4	9.3	7.7	8.2	
Seed moisture content (%)	8.5	16.9	20.3	23.5	23.3	23.2	

* = denominator of F-test is zero

72% respectively, compared to only 20% and 35% at 25°C or 30°C respectively. After 8 or 10 days accelerated ageing none of the seeds germinated at 35°C (Table 4.1).

Seedling abnormalities and ungerminated seeds increased with accelerated ageing. Aged seeds germinated at 35°C produced more abnormal seedlings and higher incidence of ungerminated seeds compared to those germinated at 25°C or 30°C. As accelerated ageing continued the tendency of the seeds to produce abnormal seedlings shifted to an increase in the number of ungerminated seeds.

It was observed that the tendency of failure to germinate occurred at an early stage of accelerated ageing when seeds were germinated at 35°C compared to germination at 25°C or 30°C. For example, seeds subjected to 8 and 10 days accelerated ageing showed more seedling abnormalities at 25°C or 30°C compared to 35°C. This was probably because most of the seeds were only capable of producing abnormal seedlings at 25°C or 30°C, whereas a germination temperature of 35°C was too high and led to germination failure in most of the seeds (Table 4.1). Poor germination of aged seeds at 35°C was reflected in higher solute leakage at 35°C. Moreover, such seeds had fewer cotyledons made up of completely living tissues, as revealed by vital staining.

The fresh weight of the seedlings in the control seed sample and seed samples aged for 2, 4, 6, 8 and 10 days decreased by about 40% when a germination test was performed at 35°C, compared to the weight of seedlings obtained from seed samples germinated at 25°C or 30°C ($P < 0.01$).

Compared to the control seed sample a noticeable decrease (35%) in seedling fresh weight at 25°C or 30°C, was observed only after the seeds had been aged for 6 days, but there was no further decrease with 8 or 10 days accelerated ageing (Table 4.1). One possible cause of decrease in seedling fresh weight could be poor seed vigour as

indicated by the poor vital staining and higher solute leakage from aged seeds. High solute leakage indicates the sensitivity of aged seeds to imbibition damage that may be contributable to a reduction in seedling fresh weight.

The conductivity of the seed soak water for the control seed sample and seed samples subject to various accelerated ageing treatments indicated that solute leakage measured by leachate conductivity was higher after incubation at 35°C, than equivalents incubated at 25°C. However, in case of seed sample aged for 8 days there was no noticeable difference in solute leakage at these two temperatures ($P = 0.3$).

Solute leakage from the seed samples at both temperatures (25°C or 35°C) was more severe in the longer accelerated ageing treatments. The seed samples showed more than 20% increase in solute leakage at 35°C compared to that recorded at 25°C ($P < 0.01$). The conductivity of aged seed samples showed that solute leakage increased at a rate directly proportional to increased ageing time and soaking temperature (Table 4.1).

4.3.2. EXPERIMENT 4.2

Action and interaction of seed moisture, temperature and time during sealed storage.

Decline in mean relative germination was rapid when seed moisture content or storage temperature was high (Table 4.2). The results indicated that a lower temperature (35° C) alone was not enough in itself for better storage; low seed moisture content (8%) was also necessary for increased life span. However, the situation varied under different combinations of seed moisture content and storage temperature. For example, seeds at 8% moisture content did not respond to a 5°C increase in storage temperature (Table 4.2). On the other hand seeds stored at 12% moisture content declined in viability when stored at 35°C ($P < 0.05$), but this decline was more rapid at 40°C ($P < 0.02$). At 35°C, seeds at 16% moisture content lost viability gradually, however, a drastic reduction in viability was observed when stored at 40°C ($P < 0.01$).

Generally there was a tendency for fewer normal seedlings and increased seedling abnormalities followed by a failure to germinate as seed moisture content, storage time and storage temperature increased (Table 4.2).

The results indicate that seed moisture, storage temperature, duration of storage and their interaction had a marked influence on normal seedlings, abnormal seedlings and ungerminated seeds ($P < 0.01$). The number of normal seedlings especially for seeds with 16% moisture content was negatively correlated with abnormal seedlings initially, but as more and more seeds died the situation reversed ($r > 0.8$).

4.3.2.1. Normal seedlings

Sealed storage at 35°C:- Mean relative percentage of normal seedlings did not decline when seed moisture content was 8% (Table 4.2). However, for seeds at 12%

moisture content the number of normal seedlings declined from 96 to 89% after 21 days ($P < 0.04$). At 16% moisture content normal seedlings rapidly declined from 96 to 80% after 7 days; 96 to 76% after 14 days and 96 to 60% after 21 days ($P < 0.03$). At 16% moisture content there was a 16% decline in the number of normal seedlings during the first week, 15% decline during the third week, but only 5% decline during the second week (Table 4.2). In other words the ability of the seeds to produce normal seedlings decreased rapidly in the initial stages of storage, followed by a steady decline and a more rapid decrease again as storage continued. This indicate that there was a great variation within the seed lot. In other words during storage some seeds lost the ability to produce normal seedlings earlier than others.

Sealed storage at 40°C:- At 8% moisture content the number of normal seedlings did not reduce after 21 days (Table 4.2). However, at 12% moisture content the number of normal seedlings decreased by 16% after 7 days; by 28% after 14 days and by 38% after 21 days ($P < 0.03$). For seeds stored at 16% moisture content the number of normal seedlings decreased drastically from 96 to 70% after 7 days; from 96 to 53% after 14 days and from 96 to only 9% after 21 days ($P < 0.01$).

At 12% moisture content the number of normal seedlings declined at the same rate during the first, second and third week (Table 4.2). At 16% moisture content the number of normal seedlings declined by 36% and 17% during the first and second week respectively and 44% during the third week of storage (Table 4.2).

4.3.2.2. Abnormal seedlings and ungerminated seeds

Sealed storage at 35°C:- The number of abnormal seedlings increased with storage time, but at a higher rate when seed moisture content was 16% compared to seeds at 8% or 12% moisture content ($P < 0.04$). Seedling abnormalities occurring from seeds at 16% moisture content increased from 4 to 15% after 7 days and from 4 to 17% after 14 or 21 days (Table 4.2).

Table 4.2. Mean data regarding normal seedlings, abnormal seedlings and ungerminated seeds in rolled paper towels after seeds were stored at three moisture contents and two storage temperartures for 7, 14 and 21 days (mean of four replications).

Observations		Storage at 35°C				Storage at 40°C			
	Ctrl	7 days	14 days	21 days	LSD at 5% (6 d.f)	7 days	14 days	21 days	LSD at 5% (6 d.f)
Normal seedlings (%)									
8% SMC	92.0	92.0	91.0	90.0	4.3	94.0	92.0	90.0	4.3
12% SMC	96.0	91.0	90.0	89.0	4.6	80.0	68.0	58.0	6.3
16% SMC	96.0	80.0	76.0	60.0	6.5	70.0	53.0	9.0	7.7
LSD at 5% (6 d.f)	6.1	5.8	5.2	7.9		5.8	6.0	6.9	
Abnormal seedlings (%)									
8% SMC	4.0	8.0	9.0	8.0	4.2	6.0	8.0	10.0	4.4
12% SMC	4.0	9.0	10.0	11.0	4.1	15.0	18.0	19.0	4.7
16% SMC	4.0	15.0	17.0	17.0	4.9	19.0	29.0	28.0	5.3
LSD at 5% (6 d.f)	3.5	5.6	3.8	5.2		4.5	4.6	5.4	
Ungerminated seeds (%)									
8% SMC	4.0	0.0	0.0	0.0	*	0.0	0.0	0.0	0.0
12% SMC	0.0	0.0	0.0	0.0	*	5.0	14.0	23.0	6.7
16% SMC	0.0	5.0	7.0	23.0	*	11.0	18.0	63.0	6.3
LSD at 5% (6 d.f)	*	*	*	*		5.6	5.3	6.1	

* = denominator of F-test is zero

At 8% or 12% moisture content the number of abnormal seedlings increased by 5% after 7 days, but did not showed any further increase after 14 and 21 days ($P > 0.2$). Seed lots at 8% or 12% moisture content did not produce any ungerminated seeds after 21 days storage (Table 4.2). However, at 16% moisture content 5% seeds were ungerminated after 7 days; 7% after 14 days and 23% after 21 days storage at 35°C ($P < 0.04$).

Sealed storage at 40°C:- The control seed lot produced 4% seedling abnormalities. When seeds were stored at 8% moisture content the number of abnormal seedlings increased by 6% after 21 days storage ($P < 0.05$). However, at 12% moisture content the number of abnormal seedlings increased by 11% after 7 days; by 14% after 14 days and by 15% after 21 days storage compared to the control ($P < 0.03$).

The control seeds at 16% moisture content produced 4% abnormal seedlings. However, seed samples at 16% moisture content produced 19%, 29% and 28% seedling abnormalities after 7, 14, or 21 days respectively ($P < 0.01$). Seeds at 8% moisture content showed 100% viability at the end of 21 days storage at 40°C (Table 4.2). However, at 12% or 16% seed moisture content high seedling abnormalities were accompanied by higher number of ungerminated seeds as storage progressed ($P < 0.03$).

Seeds at 12% moisture content showed 100% viability initially, but possessed 5% ungerminated seeds after 7 days; 14% ungerminated seeds after 14 days and 23% ungerminated seeds after 21 days ($P < 0.03$). However, those at 16% moisture content had 11% ungerminated seeds after 7 days; 18% ungerminated seeds after 14 days and as high as 63% ungerminated seeds ($P < 0.01$) after 21 days compared to none in the control (Table 4.2).

4.3.3. EXPERIMENT 4.3

Temperature, initial seed moisture and their interaction in unaged and aged seeds during germination.

The control seed lot at 12% initial moisture content and the seed lot at 16% initial moisture showed similar germinability at all the germination temperatures (Table 4.3). However, at 6% initial seed moisture content germinability tended to be lower and solute leakage was higher particularly when germination temperature was 35°C.

Seed moisture content alone did not produce any major effect on the number of normal seedlings, abnormal seedlings or ungerminated seeds when germination temperature was 17°C or 25°C (Table 4.3). At 35°C, seeds at low (6%) initial moisture content produced fewer ($P < 0.04$) normal seedlings and higher seedling abnormalities together with a higher incidence of ungerminated seeds (Table 4.3).

Accelerated ageing reduced the number of normal seedlings by 27% compared to the control ($P < 0.01$). Germination at 17°C or 25°C produced similar number of normal and abnormal seedlings or ungerminated seeds (Table 4.3), but in case of germination at 25°C, 50% germination was attained earlier ($P < 0.03$).

Germination of seeds at 35°C reduced the number of normal seedlings by 15% compared to those recorded at 25°C ($P < 0.02$). After accelerated ageing seedling abnormalities were 50% higher ($P < 0.01$). Germination at 35°C, increased the number of seedling abnormalities by 20% compared to those produced at 25°C ($P < 0.03$).

Accelerated ageing alone increased the number of ungerminated seeds by 11%, whereas seeds germinated at 35°C showed 80% more ungerminated seeds compared to those germinated at 25°C ($P < 0.01$).

Aged seed lots showed a delay of 15 h to attain 50% germination compared to unaged seed lots ($P < 0.03$). However, at 35°C, the time to 50% germination of

seeds reached 12 h sooner compared to seeds germinated at 25°C ($P < 0.02$). Solute leakage from aged seeds was 20% higher compared to the unaged seeds ($P < 0.04$). As a whole seeds soaked at 35°C leaked 23% more solutes compared to seeds soaked at 25°C ($P = 0.03$).

4.3.3.1. Normal seedlings

Unaged seed:- A general tendency of a decline in the number of normal seedlings was observed as a result of low (6%) initial seed moisture content or high (35°C) germination temperature (Table 4.3). There was very little difference in the percentage of normal seedlings between seeds at 6%, 12% (control) or 16% initial moisture content when germination occurred at 17°C ($P > 0.5$).

Seeds at either 6% or 12% initial moisture content and germinated at 25°C produced a similar number of normal seedlings (Table 4.3). The number of normal seedlings from seeds at 16% initial moisture was 5% higher at 25°C. At 35°C, seeds at 6% initial moisture content produced 10% fewer normal seedlings compared to seeds at 16% or 12% (control) initial moisture content ($P < 0.03$).

At 35°C the number of normal seedlings declined from 96% to 77% when initial seed moisture content was 6%; from 95 to 84% when initial seed moisture content was 12% and from 91 to 83% when initial seed moisture content was 16% compared to those obtained at 25°C (Table 4.3).

Aged seed sample:- At 17°C or 25°C, the number of normal seedlings in case of seeds at 6% initial moisture content was approximately similar to those produced when initial seed moisture content was 12% or 16% (Table 4.3). However, at 35°C, seeds germinated at 6% moisture content produced 12% fewer normal seedlings compared to seeds at 16% initial moisture content ($P < 0.02$).

Decline in the number of normal seedlings either as a result of low initial seed

moisture (6%) or high germination temperature (35°C) was generally more pronounced in case of aged seeds compared to the unaged seeds.

4.3.3.2. Abnormal seedlings and ungerminated seeds

Unaged seed:- There was a general tendency towards more seedling abnormalities in seeds at a lower initial moisture content (6%) or at a higher germination temperature (35°C). Seeds at 6% moisture content germinated at 17°C, recorded 12% seedling abnormalities compared to 8% abnormal seedlings at moisture contents of 12% or 16% (Table 4.3).

At 25°C, seeds at 6% or 12% moisture content produced 4% fewer abnormal seedlings than seeds at 16% moisture content (Table 4.3). At 35°C, however, seedling abnormalities were 7% higher when seed moisture content was 6% compared to seeds at 12% or 16% initial moisture content ($P < 0.04$). The minimum number of abnormal seedlings was produced at 25°C (Table 4.3). At 35°C the number of abnormal seedlings in case of seeds at 6%, 12% or 16% moisture content was 14%, 9% and 6% higher than those germinated at 25°C respectively ($P < 0.01$).

At 17°C or 25°C none of the seeds at 6%, 12% or 16% moisture content failed to germinate at 17°C or 25°C (Table 4.3). However, at 6% moisture content and 35°C ungerminated seeds increased by 5% compared to those at 25°C or 17°C ($P < 0.04$). At 12% or 16% moisture content only 2% of seedlings were abnormal at 35°C.

Aged seed sample:- There were no differences in the number of abnormal seedlings between temperatures (17°C, 25°C or 35°C) or between seeds that were at different initial moisture contents (6%, 12% or 16%). There were no differences in seedling abnormalities that could be attributed to seed moisture content because at 35°C, the seeds exhibited an increased tendency to germination failure rather than seedling abnormalities (Table 4.3).

The number of ungerminated seeds were similar at 6%, 12% or 16% moisture content incubated at 17°C or 25°C ($P < 0.05$). However, when germination occurred at 35°C, the number of ungerminated seeds obtained from seeds at 6% moisture content was 8% higher compared to those obtained when initial seed moisture content was 12% or 16% (Table 4.3).

It was observed that compared to germination at 25°C, the number of ungerminated seeds at 35°C increased, but at a higher rate when initial seed moisture content was low (6%).

4.3.3.3. Hours to 50% germination

Germination tests conducted in rolled paper towels with unaged seeds and seeds aged at low to high initial moisture contents revealed apparent differences in hours to 50% germination (Table 4.3). Germination of seeds at 16% moisture content occurred earlier. Variation between seeds at different seed moisture contents increased when germination temperature was higher (35°C) than lower (17°C). Aged seeds showed a delay in 50% germination, and this behaviour was similar regardless of initial seed moisture contents (Table 4.3).

Unaged seed:- At 17°C, seeds at 16% initial moisture content completed 50% germinated 6 h earlier than those at 6% initial moisture content ($P < 0.03$). At 17°C seeds at 12% moisture content completed 50% germination only 3 h earlier compared to seeds at 6%. Compared to seeds at 16% moisture content time to 50% germination of seeds at 12% moisture content was delayed by 3 h (Table 4.3).

At 25°C seeds at 6% or 12% initial moisture content completed 50% germination at the same time, but seeds that had an initial moisture content of 16% germinated 6 h earlier ($P < 0.03$). At 35°C seeds at 16% initial moisture content attained 50% germination 8 h earlier than seeds at 6% or 12% initial moisture content ($P < 0.02$).

Table 4.3. Mean data regarding normal seedlings, abnormal seedlings, ungerminated seeds, hours to 50% germination (mean of four replications) and conductivity (mean of two replication) in unaged and aged seed samples in rolled paper towels at different temperatures and different initial seed moisture contents.

Germination temperature	Initial seed moisture	Normal seedlings (%)	Abnormal seedlings (%)	Ungerminated seeds (%)	Hours to 50% germination	Conductivity (μSg^{-1})
Unaged seeds						
17°C	Control	92.0	8.0	0.0	97.0	
	6%	88.0	12.0	0.0	100.0	
	16%	91.0	9.0	0.0	94.0	
25°C	Control	95.0	5.0	0.0	82.0	25.8
	6%	96.0	4.0	0.0	82.0	30.6
	16%	91.0	9.0	0.0	76.0	22.1
35°C	Control	84.0	14.0	2.0	67.0	32.4
	6%	77.0	18.0	5.0	68.0	38.4
	16%	83.0	15.0	2.0	60.0	27.8
LSD at 5% (24 d.f)		7.5	6.7	*	2.2.0	3.1 (12 d.f)
Aged seeds						
17°C	Control	72.0	19.0	9.0	112.0	
	6%	69.0	24.0	7.0	115.0	
	16%	70.0	23.0	7.0	112.0	
25°C	Control	75.0	20.0	5.0	98.0	32.4
	6%	74.0	20.0	6.0	96.0	39.5
	16%	71.0	24.0	5.0	96.0	24.6
35°C	Control	54.0	25.0	21.0	74.0	41.6
	6%	47.0	25.0	28.0	75.0	44.8
	16%	56.0	25.0	19.0	69.0	34.4
LSD at 5% (24 d.f)		8.3	6.5	9.5	4.6	4.9 (12 d.f)

Control = 12% seed moisture content * = denominator of F-test is zero

Aged seed sample:- Initial seed moisture at 6%, 12% or 16% did not affect the number of hours taken to 50% germination when seeds were germinated at 17°C or 25°C. At 35°C, seeds at 16% moisture content attained 50% germination 6 h earlier compared to seeds at 6% initial moisture content. At 17°C, there was 18 h delay in time to 50% germination compared to seeds germinated at 25°C. However, time to 50% germination was delayed by 15 h when germination occurred at 25°C compared to seeds that were germinated at 35°C (Table 4.3).

4.3.3.4. Solute leakage measured by leachate conductivity

Unaged seed:- Solute leakage from the seeds soaked at 25°C was 27% or 17% higher with seeds at 6% or 12% initial moisture content respectively compared to that recorded for seeds soaked at 16% moisture content ($P < 0.04$). However, at 35°C solute leakage from the seeds soaked at 6% or 12% initial moisture content was 25% or 19% higher respectively compared to solute leakage from seeds that were soaked at 16% moisture content ($P < 0.01$).

Soaking of the seeds at 6% or 12% initial moisture content at 35°C exhibited 21% or 12% higher solute leakage, respectively compared to seeds at 16% moisture content (Table 4.3). Solute leakage from the seeds soaked at 35°C was 24% higher compared to seeds soaked at 25°C ($P < 0.01$).

Aged seed sample:- At 25°C, there was a 30% or 16% increase in solute leakage from seeds soaked at 6% or 12% initial moisture content respectively compared to seeds at 16% initial moisture content ($P < 0.02$).

At 35°C solute leakage was only 17% higher when initial seed moisture content was 12% (control), but 23% higher when initial seed moisture content was 6% compared to seeds at 16% initial moisture content ($P < 0.05$). However, in aged seeds solute leakage from seeds soaked at 35°C was only 9% higher compared to

seeds soaked at 25°C ($P < 0.01$).

4.3.4. EXPERIMENT 4.4

Effect of seed <u>etching</u> (cut on both cotyledons) on germinability of unaged and aged seed samples of three soybean cultivars.

During accelerated ageing the cultivars Essex and Pb-1, with small seeds attained a moisture content of 28.0% and 27.5%, respectively and cultivar Gemma with large seeds reached 31.9% moisture content on fresh weight basis.

The interaction of varieties, etching and ageing had a marked effect on the percentage of normal seedlings, percentage of abnormal seedlings, percentage of ungerminated seeds and solute leakage measured by leachate conductivity ($P < 0.03$).

Maximum number of normal seedlings, minimum seedling abnormalities, fewer ungerminated seeds and lowest conductivity was recorded for the smaller seeded cultivar Pb-1, followed by small seeded Essex and then the larger seeded cultivar Gemma (Figure 4.4a-c).

As a whole accelerated ageing and etching decreased the number of normal seedlings and increased seedling abnormalities or ungerminated seeds by more than 25% respectively compared to the control ($P < 0.01$). There was a negative correlation between solute leakage and the percentage of normal seedlings ($r > 0.9$) and a positive correlation between solute leakage and percentage of abnormal seedlings or ungerminated seeds ($r = 0.08$).

4.3.4.1. Normal seedlings

<u>Unaged seed</u>:- The three cultivars varied in their initial germinability. Cultivars Pb-1 and Essex with small seeds produced 99% and 93% normal seedlings respectively, and Gemma with large seeds produced 87% normal seedlings ($P < 0.03$). However, after etching variation between cultivars reduced ($P < 0.3$). This indicated that Gemma with large seeds offered comparative resistance to etching compared to

smaller seeded cultivars Essex and Pb-1. In terms of resistance to etching the Essex was superior to the second small seeded cultivar Pb-1 (Figure 4.4a). Etching reduced the number of normal seedlings by 18% in Pb-1; by 9% in Essex, but by only 7% in larger seeded cultivar Gemma compared to the controls.

Aged seed sample:- The number of normal seedlings in the case of Gemma (large seeds) was drastically reduced from 87 to 44% ($P < 0.01$), but accelerated ageing reduced the number of normal seedlings from 93 to 80% in Essex (small seeds) and from 99 to 87% in small seeded Pb-1 ($P < 0.02$). Unlike the unaged seed lots, it was the Gemma with large seeds that showed a high susceptibility to etching when aged seeds of the cultivars were tested (Figure 4.4a). In such seed lots the number of normal seedlings reduced from 80 to 67% in Essex (small seeds); from 44 to as low as 19% in Gemma (large seeds) and from 87 to 60% in Pb-1 (small seeds).

These results indicated that high initial germinability may not be an indication of the resistance of a cultivar to etching, however, the susceptibility of a cultivar to accelerated ageing may well reflect its susceptibility to etching.

In Gemma (large seeds) and Essex (small seeds) accelerated ageing had a more dominant effect in decreasing the number of normal seedlings compared to etching (Figure 4.4a). However, in cultivar Pb-1 (small seeds) etching reduced the number of normal seedlings at a higher rate compared to accelerated ageing.

4.3.4.2. Abnormal seedlings and ungerminated seeds

Unaged seed:- Compared to the control etching increased the number of abnormal seedlings irrespective of the cultivar ($P < 0.03$). However, the extent of the effect was influenced by variety (Figure 4.4b). Initially abnormal seedlings in Essex (small seeds) and Gemma (large seeds) were very low. Etching increased the number of abnormal seedlings from 0 to 11% in cultivar Pb-1 ($P < 0.01$). In Essex (small seeds),

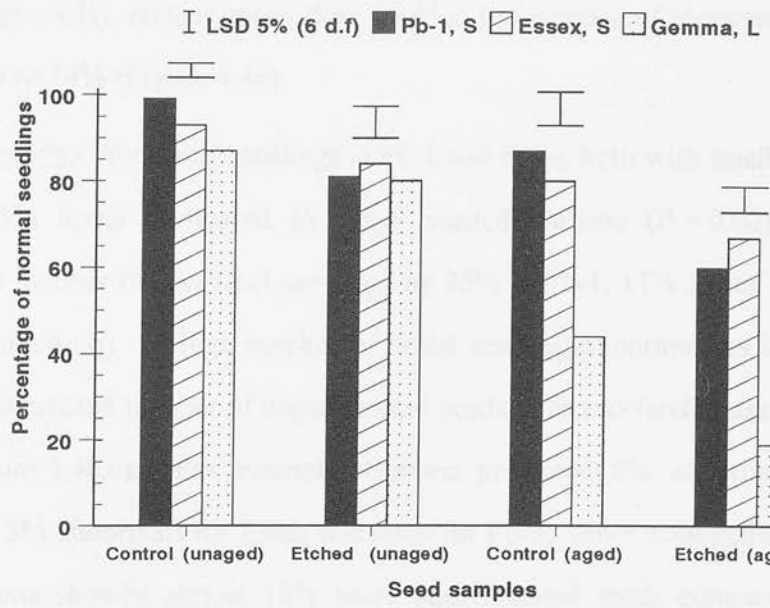


Figure 4.4a. Effect of etching on the percentage of normal seedlings in unaged and aged seeds of three soybean cultivars (S) small seeded, and (L) large seeded.

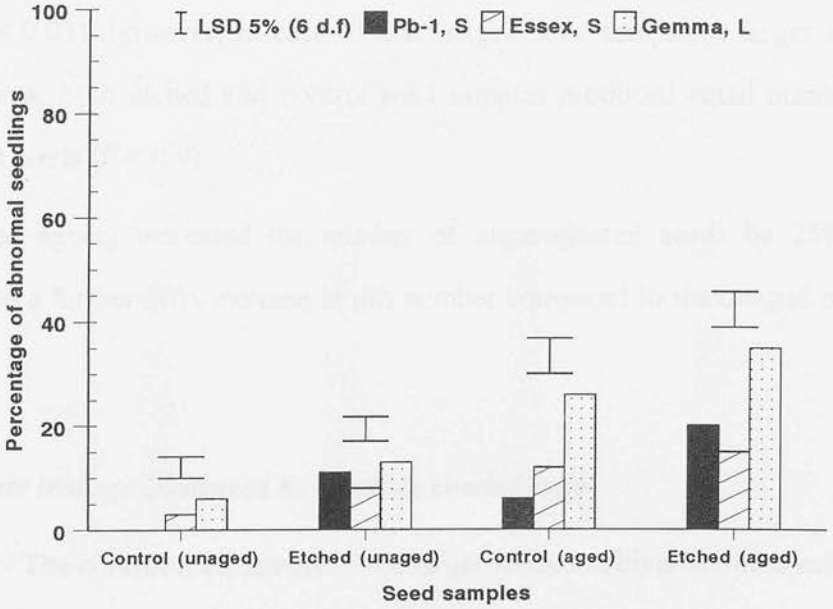


Figure 4.4b. Effect of etching on the percentage of abnormal seedlings in unaged and aged seeds of three soybean cultivars (S) small seeded, and (L) large seeded.

etching increased seedling abnormalities from 4 to 8% ($P < 0.02$). However, in Gemma (large seeds), etching more than doubled the number of abnormal seedlings, i.e. from 6% to 14% (Figure 4.4b).

Aged seed sample:- Abnormal seedlings in Pb-1 and Essex both with small seeds were 25% and 11% lower compared to larger seeded Gemma ($P = 0.02$). Etching increased the number of abnormal seedlings by 25% in Pb-1; 11% Essex and 21% in Gemma (Figure 4.4b). A high number of initial seedling abnormalities in a cultivar indicated an increased number of ungerminated seeds after accelerated ageing or after etching (Figure 4.4b,c). For example, Gemma produced 6% abnormal seedlings compared to 3% abnormals for Essex and none for Pb-1. After accelerated ageing or etching Gemma showed almost 12% more ungerminated seeds compared to aged seeds of Essex and Pb-1 (Figure 4.4c).

There was a great difference in the number of ungerminated seeds between cultivars, between unaged and aged seed samples and between etched and control seed lots ($P < 0.03$), however, in case of the unaged seed sample of larger seeded cultivar Gemma, both etched and control seed samples produced equal numbers of ungerminated seeds ($P = 0.9$).

Accelerated ageing increased the number of ungerminated seeds by 25% and etching caused a further 26% increase in this number compared to the unaged control ($P < 0.01$).

4.3.4.3. Solute leakage measured by leachate conductivity

Unaged seed:- The control seed sample of the larger seeded cultivar Gemma exhibited 11% higher solute leakage compared to control seed sample of small seeded cultivars Essex and Pb-1 ($P < 0.03$). Moreover, Essex leaked 7% more solutes compared to Pb-1. Etching markedly increased solute leakage in Gemma ($P < 0.04$), but there was

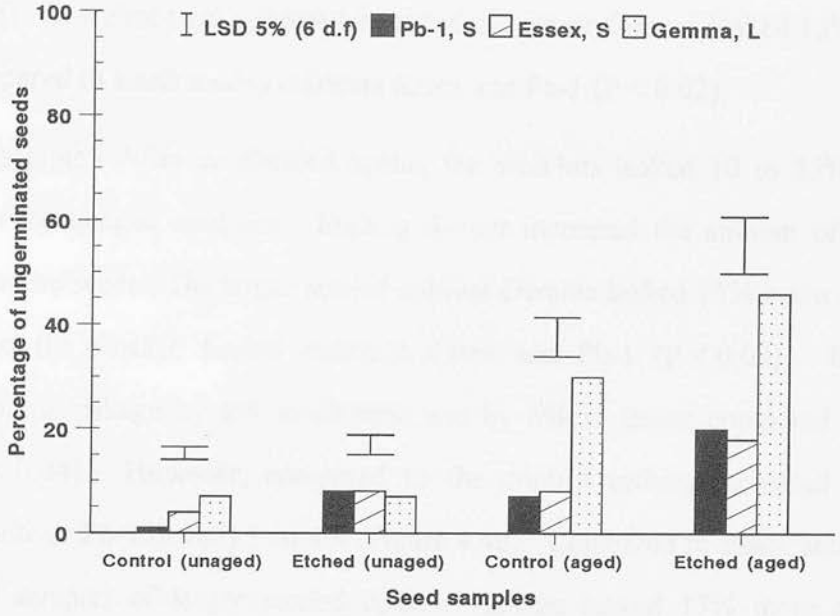


Figure 4.4c. Effect of etching on the percentage of ungerminated seeds in unaged and aged seeds of three soybean cultivars (S) small seeded, and (L) large seeded.

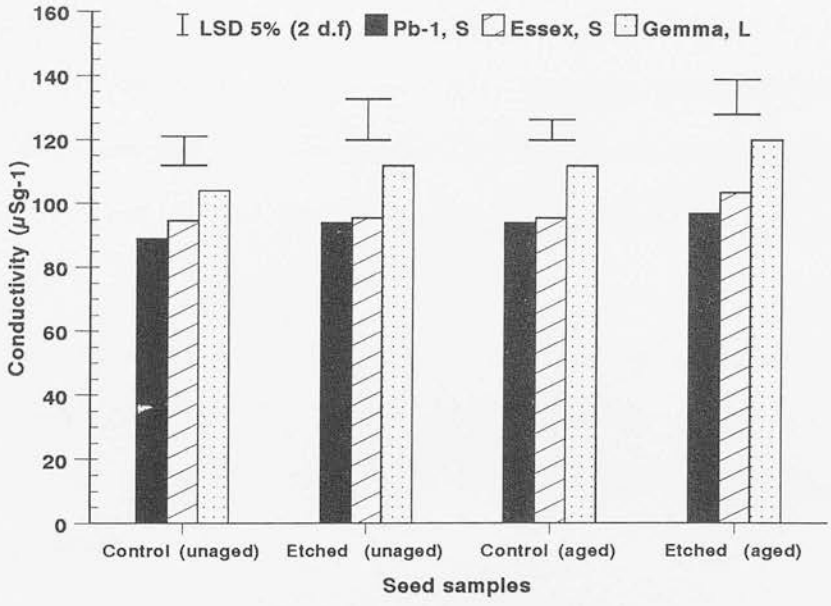


Figure 4.4d. Effect of etching on conductivity of soak water in unaged and aged seeds of three soybean cultivars (S) small seeded, and (L) large seeded.

little increase in solute leakage from small seeded cultivars Essex and Pb-1 (Figure 4.4d). In etched seed samples large seeded cultivar Gemma leaked 13% more solutes compared to small seeded cultivars Essex and Pb-1 ($P < 0.02$).

Aged seed sample:- After accelerated ageing the seed lots leaked 10 to 25% more solutes than the unaged seed lots. Etching further increased the amount of solute leakage from the seeds. The larger seeded cultivar Gemma leaked 15% more solutes compared to the smaller seeded cultivars Essex and Pb-1 ($P < 0.03$). Etching increased solute leakage by 8% in Gemma and by 6% in Essex compared to the control ($P < 0.04$). However, compared to the control etching increased solute leakage in cultivar Pb-1 by only 3 to 4% (Figure 4.4d). Compared to Essex and Pb-1, etched seed samples of larger seeded cultivar Gemma leaked 13% more solutes ($P < 0.02$).

4.4. DISCUSSION

Several scientists have examined seed lots of a large number of soybean (*Glycine max* L. Merrill) cultivars and repeatedly correlated the tetrazolium topographic staining, conductivity and standard germination tests to field emergence (Yaklich & Kulik, 1979).

The results of experiment 4.1 agree to the findings of these scientists to some extent. However, it was further revealed that the laboratory tests, especially the tetrazolium topographic staining over estimated the field emergence capacity of low vigour seed samples. The reason for this result with tetrazolium chloride may be the capability of weakly living parts of the seed to stain, but unable to cope with the complex physiological and biochemical processes of germination.

The work of Tekrony & Egli (1977) supports the present results. They believe that to achieve a reliable estimation of field emergence, dependence on a single viability test is not sufficient. Therefore, different tests should be utilised especially if seeds are to be planted in adverse soil conditions. However, they found large differences in the field emergence of seed lots with high levels of laboratory germination and confirmed that laboratory tests were inadequate as a mean of identifying seed lots that may perform poorly under field conditions.

These results, however, showed that laboratory tests can be a good indicator of field emergence in case of seed samples showing a laboratory germination of 80% or more. However, these tests fail to provide a satisfactory estimation of field emergence in seed samples producing less than 80% normal seedlings under laboratory conditions.

Tekrony & Egli (1977) have also suggested that in fact laboratory tests provides optimum conditions for seed germination and this is the major weakness of these tests when trying to estimate field emergence. Unlike those in the laboratory, conditions in

the field are far from ideal.

The conductivity results indicated that higher solute leakage occurred as temperature increased, and that seed leakage was further exacerbated by ageing. A similar conclusion was made by Powell *et al.* (1984). Schoettle & Leopold (1984) also reported high solute leakage from aged soybean seeds. The field emergence test in Pakistan was performed during the hot month of July. High alternate day (35°C to 40°C) and night (15°C to 20°C) soil temperatures in the field may have caused severe seed damage and promoted high leakage particularly from the lower vigour seeds.

In the standard germination test the seedlings achieved their maximum fresh weight, when germination occurred at 30°C. This confirms the results of research workers who have reported 30°C as the ideal germination temperature for rapid growth of soybean (Brown, 1960; Tyagi & Tripathi, 1983; Bharati *et al.*, 1983). Hatfield & Egli (1974) reported maximum hypocotyl elongation at 30°C in Cutler and Lee soybeans. When germination occurred at 25°C or 30°C (experiment 4.1) seeds aged for 2 or 4 days did not show reduced seedling fresh weight even though at 25°C the aged seeds showed higher solute leakage compared to the unaged control. This has been shown by Powell & Matthews (1980) who reported that in peas solute leakage increased before reduced performance was detectable.

Seedling fresh weight in experiment 4.1 decreased by 50% at 35°C compared to that at 25°C. These results are consistent with those obtained by Emerson & Minor (1979) who observed a 78.8% decrease in seedling length of soybean when subject to 38°C for 20 h to 24 h.

The results also showed that germination temperature beyond certain limits can alone cause massive damage to seed viability and seedling vigour. This is especially evident if the seed had already deteriorated due to a poor storage environment. High

solute leakage was associated with reduced number of normal seedlings and a decrease in seedling fresh weight. This indicates that germination conditions that encourage high solute leakage may discourage normal germination probably due to adverse effects on physiological and biochemical processes.

High solute leakage means loss of membrane integrity and loss of membrane integrity lead to physiological and biochemical incompetence in germinating seed. These conclusions are in consistent with a review on seed vigour and seedling establishment by Powell (1988). Unfortunately, low seed vigour and adverse planting conditions (high temperature) are common in most of the potential soybean growing areas in Pakistan.

The results of experiment 4.2 revealed that seeds stored at different seed moisture contents, deteriorated at different rates. This relationship had been established earlier by James (1967). Seeds in each sample developed into normal or abnormal seedlings or failed to germinate. Research workers have reported that the potential of a seed sample to maintain viability during storage varies between different seed lots of the same cultivar and between seeds within a seed lot. Moreover, the seeds probably lost their ability to germinate followed by a total germination failure. Similar results have been reported by Roberts (1972). Moreover, these results (experiment 4.2) revealed that the rate of loss of seed viability depended mainly upon seed moisture and storage temperature, whereas the length of storage determined the extent of variation within a seed sample.

When seed moisture was higher (16%) and storage temperature was low (35°C) most of the seeds lost viability during the third week of storage, however, at 12% seed moisture content and 40°C storage temperature seed viability declined gradually during the first, second and third week. This means that the behaviour of seed deterioration varies with variation in storage conditions. Seeds at 12% or 16% moisture content lost viability quicker than those at 8% moisture content. This was to

be expected as previous research work has shown quicker viability loss when seeds were stored at higher moisture content (Ellis, 1982).

The decisive role in loss of normal germination varied with different combinations of these two environmental factors seed moisture content and storage temperature. For example, seeds at 12% moisture content maintained viability as well as seeds at 8% moisture at 35°C. But a rapid decline in seed viability occurred at 12% moisture content at 40°C. Under these conditions storage temperatures played a decisive role in viability loss. Conversely seeds at 8% moisture content were not affected by either 35°C or 40°C or by duration of storage. Therefore, in this case, it was the moisture content of the seeds that had a decisive role in maintenance of seed viability. Harrington and Douglas (1970) have previously established the importance of seed moisture content and storage temperature. The results of experiment 4.2 further confirmed that the two prime factors responsible for the loss of seed vigour and viability in tropical and subtropical regions are adverse storage temperatures and poor drying of seeds by farmers so that the moisture content of the seeds is low enough for safe storage.

Due to the predominantly poor economic conditions in third world countries, storage of the seeds at low temperatures (between 5°C to 10°C) in cold stores is not available to farmers (Harrington & Douglas, 1970). In experiment 4.2 unfavourable storage conditions rapidly reduced seed quality, impaired germination and normal seedling growth. Similar results were shown by Ching & Schoolcraft (1968).

Sealed storage at low moisture content (8%) and low temperature (35°C) improved storage life. Therefore, it can be suggested that cold stores may not be necessary if the seeds are properly dried to a moisture content of 8% or less before storage and stored in sealed containers. Sealed storage at even lower (6%) seed moisture content should maintain high seed vigour and viability during the 8 to 9 months storage period in the plains of Pakistan.

It has already been reviewed that seeds at low initial moisture are vulnerable to soaking injury. According to the results obtained in experiment 4.3, reduction in the percentage of normal seedlings of seeds at 6% moisture content was greater at 35°C. This was attributed to rapid water uptake, causing higher solute leakage. Lower initial seed moisture content was associated with high solute leakage, but did not necessarily accompany a decline in normal germination if germination temperature is low (25°C or 30°C).

The negative effect of germination temperature (35°C) on seeds at low moisture content was more severe in aged seeds. This may be due to reduced respiratory activity in aged seeds leading to a reduced activity of essential catabolic and anabolic pathways of lower compared to higher quality soybean seeds (Wahab & Burris, 1971). The initial moisture content of the seeds appeared to make a substantial difference to the number of normal seedlings only if germination temperature was high (35°C), but was not so apparently important at 17°C or 25°C.

These results (experiment 4.3) agree to the conclusions drawn by previous research workers (Hobbs & Obendorf, 1972; Bartsch *et al.*, 1986) who reported that the initial moisture content of the seeds played a vital role in determining the amount of solute leakage and success in germination especially when germination temperature was high. The current results also suggested that minor increases in the leakage of solutes may be associated with germination failure or at least with an increase in seedling abnormalities, particularly in case of some of the potentially low vigour seeds within a seed sample. However, there was a tendency towards germination failure and an increase in the number of abnormal seedlings regardless of whether the increase in solute leakage was due to poor seed vigour, low initial seed moisture, high imbibition temperature or due to interaction of these factors. Koslanund & Delouche (1987) associated high solute leakage with reduced germinability when imbibition temperature was high (35°C or more).

Loeffler *et al.* (1988) reported that seed moisture contents ranging from 11% to 18% did not significantly increase seed leachate conductivity. The findings of experiment 4.3 do not completely agree to his claim. The current results have shown that there was a significant difference between solute leakage of seeds at 12% or 16% initial seed moisture content if low vigour seeds were used, or if the conductivity test was conducted at 35°C rather than at 25°C. Therefore, it is suggested that a conductivity test should not be necessarily conducted at 25°C. This is because the behaviour of solute leakage varies with soaking temperature and is also dependent upon seed quality.

If a conductivity test is used to measure the vigour level of a seed sample for known field planting conditions, then the test should consider seed quality, initial seed moisture content and soil temperature to ensure that solute leakage from a seed sample in the laboratory is a good indicator of its performance under field conditions.

In experiment 4.3 seeds at low moisture content (6%) exhibited higher initial solute leakage and decreased germinability, particularly when germination temperature was high (35°C) or if aged seeds were used. These findings are supported by Parrish & Leopold (1978) who reported similar results after testing soybean seeds at different initial moisture contents.

The seed coat plays a vital role in regulating the uptake of moisture from a high relative humidity environment or in the early phases of rapid imbibition and thus determine storage life and the extent of soaking injury (Calero *et al.*, 1981). Experiment 4.4 showed that different soybean cultivars of high initial germination deteriorated at different rates. Due to application of accelerated ageing cultivars varied in their ability to retain organic leachates during the process of imbibition. It was observed that both accelerated ageing and seed etching decreased the number of normal seedlings and increased seedling abnormalities and ungerminated seeds, but this phenomenon varied between cultivars.

Cultivars differed significantly for the conductivity of leachate water recorded after 4 h, however, there were very little differences in the conductivity of soak water that could be related to accelerated ageing, probably, because 4 h was too long a soaking time to detect differences in seed samples. This can be explained, because soybean seeds imbibe water rapidly in the first 30 minutes, the rate then slows down to a steady imbibition. Parrish & Leopold (1977) called this initial inrush of water to cause severe soaking injury, particularly to aged seeds. Moreover, the seed coat of soybean delays water uptake during the first few hours of soaking and eventually serves as a reservoir of water for the seeds (McDonald *et al.*, 1988b).

As a result of experiment 4.4 it is recommended that when comparing soybean seed samples or varieties a conductivity measurement should be made within the first 30 minutes, so that high vigour seeds can show their superiority in this regard. Simon & Raja Harun (1972) also support these findings. They reported that extensive leakage from seeds persists for only minutes after which membranes recover their normal selective permeability. Probably the recovery mechanism partly repaired the damage caused by ageing.

According to the results obtained by Bewley (1984) deteriorated seeds do leak significantly more respirable substrates compared to high quality seeds. Results of experiment 4.4 indicated that solute leakage is a complex and unpredictable mechanism. Therefore, a single conductivity test under certain specified set of conditions cannot define the overall mechanism of solute leakage. The pattern of solute leakage varies and is dependent on prevailing conditions and varies with cultivar. All cultivars tested were significantly damaged by etching, but etched seed sample of the small seeded type Pb-1 were more sensitive. Researchers have reported reduced germination and a high number of abnormal seedling and ungerminated seeds in aged (Oliveira *et al.*, 1984) or etched seeds (Burchett *et al.*, 1985).

Vanangamudi (1988) reported that cultivars with small seeds retained their viability

better during accelerated ageing. The small seeded cultivar (Pb-1) leaked less organic solutes than Gemma (large seeds). This means that the cultivar Pb-1 with small seeds exhibited the impermeable response at a higher rate as compared to Gemma. Hill *et al.* (1986b) agree to these findings. Hill *et al.* (1986b) found a negative relationship between seed size and impermeable seed expression in soybean. The results of this study suggest that high initial seed quality is a prerequisite of good storability, but will not in itself guarantee an adequate storage life under conditions of high relative humidity and high temperature. Of the characteristics considered the seed coat appears to offer the most promise for use in the improvement of soybean storability under tropical and subtropical conditions of high temperature and high relative humidity. This was shown by minimum solute leakage and maximum germinability observed in case of small seeded cultivar Pb-1 that possessed a waxy type tough seed coat compared to large seeded cultivar Gemma, that had a rough seed coat.

CHAPTER 5

EVALUATION OF WATER UPTAKE INJURY AND MOISTURE STRESS USING POLYETHYLENE GLYCOL (PEG 8000)

5.1. INTRODUCTION

The role of PEG as an osmo conditioner has been acknowledged by many workers (Mukherji & Dey, 1985; Seong, 1986; Seong *et al.*, 1988; Murray, 1989). According to these workers imbibition injury can be prevented by slowly hydrating the seeds in PEG.

It has been reported by Parrish & Leopold (1977) that in the initial stages of imbibition soybean seeds absorb water at a higher rate that aggravate the extent of soaking injury caused to the seeds. Similarly Powell & Matthews (1978) and Saha & Basu (1982) concluded that in peas (*Pisum sativum*) low vigour seeds experienced more imbibition injury compared to high vigour seeds. Parrish & Leopold (1977) further reported that the initial inrush of water into the soybean cotyledons is difficult to control.

In the tropics and subtropics soybean planting is frequently followed by heavy rains which result in poor crop stands due to severe soaking injury and anoxia. Shanmugasundaram (1980) reported a 28% reduction in soybean plant establishment due to flooding that occurred immediately after sowing.

A laboratory experiment based on soaking of unaged and aged seed lots in water and different concentrations of PEG may well reflect the extent of damage that could be caused by short term or long term flooding. This type of approach may show if

soaking injury or anoxia play a decisive role under a specific length of saturated conditions.

Many research workers have been successful in controlling soaking injury in soybean seeds through slow imbibition in PEG (Mukherji & Dey, 1985; Seong et al., 1988). It has been confirmed that the rate of water absorption particularly in case of low vigour seeds plays a vital role in germination success of soybean (Tilden and West, 1985). However, whether a pre-planting treatment consisting of slow imbibition of soybean seeds to a certain pre-determined seed moisture content either in water or various concentrations of PEG can enhance or improve germination need further investigation.

Studies have shown that the water uptake injury that occurs in isolated embryonic axes during the initial period of imbibition particularly in low vigour soybean seeds can be prevented by osmotic control of water uptake (Woodstock & Taylorson, 1981). However, there is a need to evaluate the optimum concentration of PEG to serve as a medium for pre-planting imbibition of soybean seeds.

Enhancing the rate of germination to get the seedlings out of the soil as early as possible or before crust formation, is a priority in tropical and subtropical soybean cultivation (Dadson, 1982). In tropical and subtropical regions temperatures much higher than optimum (35°C to 42°C) have been reported by Emerson (1982). Such conditions encourage severe damage to germinating seeds and due to rapid evaporation the soil lose moisture before acceptable emergence is attained. Under those conditions most of the seeds germinate, but due to limited moisture stress the seedlings are trapped within the soil structure.

Alongside the exploitation of the genetic variability of soybean cultivars (Andrews, 1982) there is a possibility of improving or enhancing germination if seeds are partially imbibed before germination. Such practice may contribute to success in

improved emergence by allowing the seeds to cut down the time taken to emergence under stressful planting conditions.

There is also a lack of focus on the study of the direct effect of slow imbibition in PEG on early seedling growth. In tropical and subtropical countries soybean seeds experience waterlogged conditions due to rainfall immediately after planting which can result in severe soaking injury and poor crop stands (Troedson *et al.*, 1983). Moreover, under tropical and subtropical conditions seeds frequently suffer from moisture stress, because soil moisture under these conditions does not cope with emergence of the seedlings (Dharmasena, 1983). Enhanced germination under tropical and subtropical conditions could probably enable the seeds to emerge and establish before moisture stress occur. The availability of moisture for seed imbibition relies on the composition of the medium in which germination takes place. This is of special significance under natural conditions where the solution in which the seeds imbibe is not pure water. As the concentration of solutes in a solution increase, imbibition may decrease, largely due to osmotic effects. Soybean seeds require an ample supply of moisture for successful germination. However, the rate of water uptake plays a vital role in success of germination. The results of an experiment performed by Peske (1983) suggested that entry of water into the seeds is not only determined by its availability in the germination medium, but also by the permeability of the seed coat.

Aged seeds or heat killed soybean seeds are reported to imbibe water more rapidly than high vigour seeds. Water uptake into soybean seeds particularly those of low initial vigour must be regulated, otherwise the seed suffers from severe soaking injury, especially, if the rate of water absorption is high. Seeds may fail to germinate if the available moisture is limiting.

Results regarding the effects of moisture stress are conflicting. Some workers believe that seed germination is more sensitive to water stress than subsequent growth

(Sumarno, 1986). Though it is not very ideal to interpret the results of laboratory experiments on water uptake with the behaviour of seeds in field. However, laboratory experiments provide sufficient information to further monitor this relationship in the field in the most desirable way.

Keeping in mind the above literature it was concluded to evaluate the effect that slow imbibition and osmotic stress have on germinability and speed of germination in unaged and aged seeds.

5.2. SPECIFIC METHODS

5.2.1. Experiment 5.1

Comparative damage caused to high or low vigour soybean seeds soaked in distilled water or polyethylene glycol (PEG) before a germination test.

A seed sample of low initial vigour was obtained by subjecting uniform and healthy looking seeds of soybean cultivar Epps to accelerated ageing for 3 days in a sealed desiccator placed in a water bath at 41°C (between 95% and 100% relative humidity). A separate batch of unaged seed was also used.

Both the unaged and aged seed samples were allowed to equilibrate to a uniform moisture content at room temperature for 4 days. The seeds were either soaked for 5 h or 10 h in distilled water or 10%, 25% or 50% PEG at 20°C. Unaged and aged batches of seeds that were not soaked were the control. Before germination the seeds soaked in PEG and the control seeds were imbibed for 3 to 10 h between paper towels to equilibrate with the seeds soaked in distilled water.

A germination test was conducted in the greenhouse in pots containing compost (12°C to 20°C). Sowing depth was 2.5 cm. Before the germination tests the seeds were treated with 1% sodium hypochlorite for 5 seconds. Four replications of 20 seeds each were used. Data were recorded on the number of normal seedlings, number of abnormal seedlings, number of ungerminated seeds and days to 50% emergence. Details are given in chapter 3.

5.2.2. Experiment 5.2

Elevating soybean germination by pre-planting slow imbibition of unaged and aged seeds in polyethylene glycol (PEG).

Healthy seeds of the cultivar Essex were subjected to accelerated ageing for 3 days as in experiment 5.1. An unaged seed sample was the control treatment. Both the

unaged and aged seed samples were equilibrated to a uniform moisture level of about 10% at room temperature.

The seeds were treated with Dithane M-45. Three layers of 6" x 6" paper towel were uniformly applied with distilled water and 10%, 25%, 35% and 50% PEG. The paper towels were squeezed between a ruler and smooth table surface in the laboratory to ensure that the paper towels had been uniformly applied.

Seeds were placed on a double layer of paper towel, and covered with another single layer. The paper towels were then covered with aluminium foil to control evaporation. In this way weighed seed samples of both the aged and unaged seeds were allowed to imbibe for 24 h at 20°C.

After incubation three replications of 25 seeds each per treatment were germinated in rolled paper towels moistened with distilled water. The paper towels were placed in incubators in dark at 25°C. Seeds were examined 2 to 3 times daily to record the number of hours taken to 50% germination. Data on the number of normal or abnormal seedlings and ungerminated seeds were recorded after 8 days. The number of hours taken to 50% emergence was also recorded. Details are given in chapter 3.

5.2.3. Experiment 5.3

Minimising soaking injury by slow imbibition of high and low vigour soybean seeds in 25% polyethylene glycol (PEG) before germination.

Healthy looking seeds of uniform size of the cultivar Epps and Stafford were used. Seeds were exposed to accelerated ageing for 2 and 4 days as in experiment 5.1. An unaged seed sample was used as the control. The seeds were imbibed for 24 h in rolled paper towels uniformly applied with distilled water (control) or 25% PEG at room temperature at 20°C.

The seeds were treated for 5 seconds in 1% sodium hypochlorite and a germination

test was conducted in compost in the growth room (25°C; 12 h/d photoperiod) using three replications 25 seeds each. After 10 days of planting data were recorded on the number of normal and abnormal seedlings, number of ungerminated seeds, shoot length, shoot fresh weight and shoot dry weight (grams). Details are given in chapter 3.

5.2.4. Experiment 5.4

Effect of osmotic stress applied through different concentrations of polyethylene glycol (PEG) on germinability of unaged and aged seeds.

Seeds of the cultivar Epps aged in a sealed desiccator for 3 days as in experiment 5.1. An unaged batch of seed was the control. Both the high and low vigour seed samples were equilibrated to a uniform moisture level of 10% at 20°C.

Four replications of 25 seeds each were treated for 5 seconds in 1% sodium hypochlorite and then germinated in incubator at 25°C in dark, in rolled paper towels uniformly supplied with 0%, 5%, 10%, 15%, 20%, or 25% PEG. After 10 days of germination data were recorded on the number of germinated seeds and seedling fresh weight (grams).

Two replications of 25 seeds were weighed and then imbibed for 24 h between paper towels supplied with distilled water or PEG at 20°C. The seeds were removed after 24 h, surface cleaned with tissue paper and weighed again (W2). Percentage increase in seed moisture was calculated. Details are given in chapter 3.

5.3. RESULTS

5.3.1. Experiment 5.1

Comparative damage caused to high or low vigour soybean seeds soaked in distilled water or polyethylene glycol (PEG) before a germination test.

The unaged (control) seeds produced 90% normal seedlings, 6.5% abnormal seedlings and 3.5% ungerminated seeds. However, after ageing 62% normal seedlings, 23% abnormal seedlings and 15% ungerminated seeds were produced (Figure 5.1a-f). The unaged control seed sample required 5.8 days to attain 50% emergence, whereas the aged (control) seed sample took 7.5 days to emergence (Figure 5.1g,h).

The polyethylene glycol concentration of soak solution and the duration of soaking had a significant effect on the number of normal seedlings for both unaged and aged seed samples ($P < 0.01$).

Germinability and growth of both unaged and aged seeds was markedly reduced by soaking in distilled water for 5 or 10 h (Figure 5.1a,b,g,h). However, unaged and aged seeds assessed after soaking in distilled water and various concentrations of polyethylene glycol revealed marked differences in their response to slow imbibition in PEG and to the duration of soaking.

Soaking of aged seeds for 5 h in either 25% or 50% PEG could only maintain the initial high number of normal seedlings, but did not improve germination (Figure 5.1b). This clearly shows that polyethylene glycol can play a crucial role in reducing water uptake injury in aged seeds. In no case did soaking in 25% PEG reduced germination compared to soaking in distilled water or 10% PEG; likewise in no case did the growth of seeds soaked in 25% PEG exceed those from the controls. Soaking in PEG probably prevented abnormal growth caused by accelerated ageing.

In aged seeds the number of normal seedlings obtained after soaking for 5 h in

PEG or distilled water was 13% higher ($P < 0.04$) compared to normal seedlings obtained after 10 h soaking. Accelerated ageing decreased the number of normal seedlings by 28% ($P = 0.01$), however, seedling abnormalities and ungerminated seeds increased by 16.5% or 11.5% respectively ($P < 0.03$).

The number of normal seedlings decreased at a higher rate when soaking occurred in distilled water, but soaking in PEG increased the chances of a seed producing a normal seedling compared to a seed soaked in distilled water. The tendency to increase the number of normal seedlings increased as the concentration of PEG increased (Figure 5.1a,b). Increase in the number of normal seedlings could be attributed to reduced soaking injury in PEG compared to distilled water.

5.3.1.1. Normal seedlings

Unaged seeds soaked for 5 h:- The number of normal seedlings produced after soaking in water were 39% less than the control. This situation was progressively improved as seeds were soaked in increasing concentrations of PEG (Figure 5.1a). After soaking in 25% or 50% PEG seeds produced 17% more normal seedlings compared to those obtained after soaking in 10% PEG ($P < 0.01$). Soaking in 10% PEG produced 12% ($P = 0.02$) more normal seedlings as compared to soaking in distilled water (Figure 5.1a).

Unaged seeds soaked for 10 h:- Soaking in distilled water and 10%, 25% or 50% PEG decreased the number of normal seedlings by 55%, 45%, 24% or 23% respectively compared to the unaged control ($P < 0.01$). The number of normal seedlings produced after soaking in 25% or 50% PEG were 21% higher than those obtained after soaking in 10% PEG ($P < 0.01$). However, after soaking in 10% PEG, 10% more normal seedlings were produced compared to those produced after imbibition for 10 h in distilled water ($P = 0.01$).

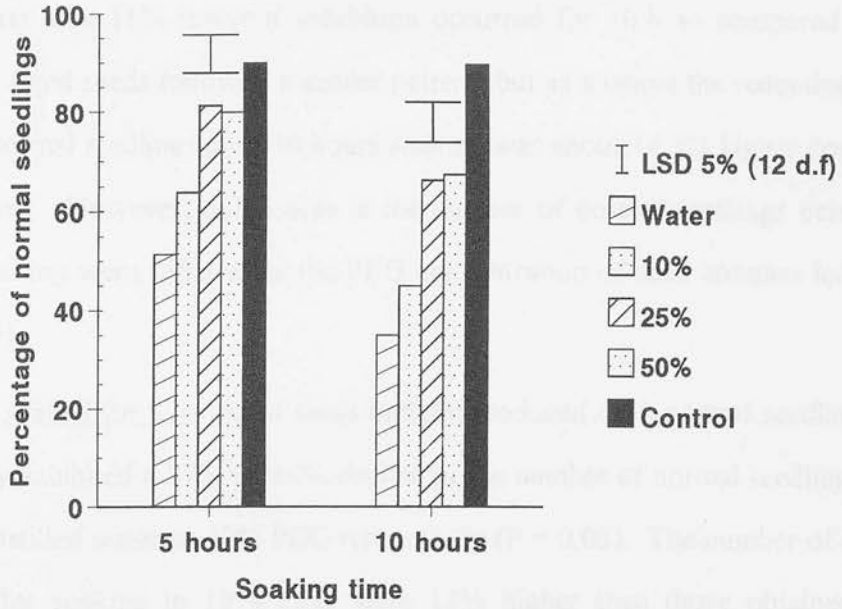


Figure 5.1a. Comparative effect of pre planting soaking in distilled water or polyethylene glycol (%) on the number of normal seedlings in unaged seeds of cultivar Epps.

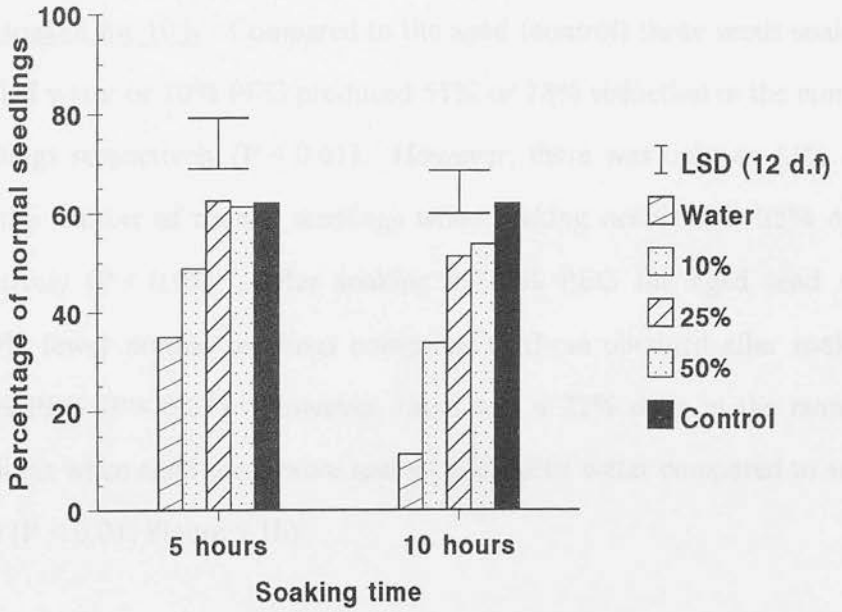


Figure 5.1b. Comparative effect of pre planting soaking in distilled water or polyethylene glycol (%) on the number of normal seedlings in aged seeds of cultivar Epps.

In unaged seeds the number of normal seedlings produced after soaking in PEG or distilled water was 11% lower if imbibition occurred for 10 h as compared to 5 h ($P < 0.02$). Aged seeds followed a similar pattern, but as a whole the reduction in the number of normal seedlings after 10 hours soaking was about 14.5% higher compared to 5 h soaking. However, differences in the number of normal seedlings between 5 and 10 h soaking were reduced as the PEG concentration of soak solution increased (Figure 5.1a).

Aged seeds soaked for 5 h:- Aged seeds initially produced 62% normal seedlings and subsequently exhibited a 27% or 14% decline in the number of normal seedlings after soaking in distilled water or 10% PEG respectively ($P = 0.03$). The number of normal seedlings after soaking in 10% PEG were 13% higher than those obtained after imbibition in distilled water ($P < 0.01$). However, soaking in 25% or 50% PEG for 5 h maintained the initial number of normal seedlings produced by the aged seed sample (Figure 5.1b).

Aged seeds soaked for 10 h:- Compared to the aged (control) those seeds soaked for 10 h in distilled water or 10% PEG produced 51% or 28% reduction in the number of normal seedlings respectively ($P < 0.01$). However, there was only an 11% or 9% decrease in the number of normal seedlings when soaking occurred in 25% or 50% PEG respectively ($P < 0.05$). After soaking in 10% PEG the aged seed sample exhibited 19% fewer normal seedlings compared to those obtained after soaking in 25% or 50% PEG ($P < 0.01$). However, there was a 22% drop in the number of normal seedlings when aged seeds were soaked in distilled water compared to soaking in 10% PEG ($P < 0.01$; Figure 5.1b).

5.3.1.2. Abnormal seedlings and ungerminated seeds

Polyethylene glycol concentration or soaking duration had an effect on seedling abnormalities in both the unaged or aged seed samples ($P < 0.01$). Aged seeds

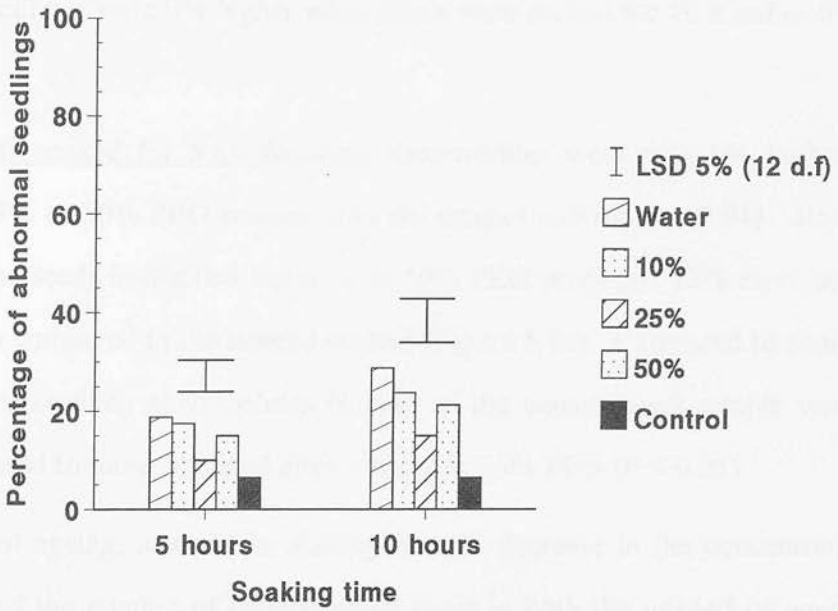


Figure 5.1c. Comparative effect of pre planting soaking in distilled water or polyethylene glycol (%) on the number of abnormal seedlings in unaged seeds of cultivar Epps.

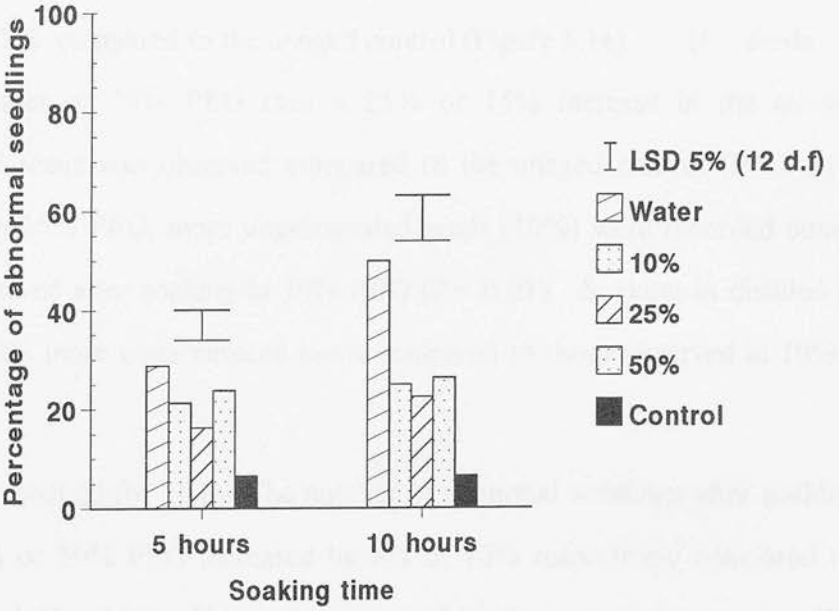


Figure 5.1d. Comparative effect of pre planting soaking in distilled water or polyethylene glycol (%) on the number of abnormal seedlings in aged seeds of cultivar Epps.

produced 8% more abnormal seedlings compared to the unaged seeds (Figure 5.1c,d). Abnormal seedlings were 9% higher when seeds were soaked for 10 h rather than 5 h ($P < 0.01$).

Unaged seeds soaked for 5 h:- Seedling abnormalities were only 6% higher after soaking in 25% or 50% PEG compared to the unaged control ($P < 0.01$). However, soaking of the seeds in distilled water or in 10% PEG produced 12% more seedling abnormalities compared to the unaged control (Figure 5.1c). Compared to soaking in distilled water seedling abnormalities in case of the unaged seed sample were 6% higher compared to those obtained after soaking in 25% PEG ($P < 0.05$).

Accelerated ageing, increase in soaking time or decrease in the concentration of PEG increased the number of ungerminated seeds in both the unaged or aged seed samples ($P < 0.01$; Figure 5.1e,f).

In 50% PEG no increase in the incidence of ungerminated seeds occurred compared to the unaged control. In 25% PEG, the number of ungerminated seeds increased by 5% compared to the unaged control (Figure 5.1e). If seeds were soaked in water or 10% PEG then a 25% or 15% increase in the number of ungerminated seeds was observed compared to the unaged control ($P < 0.01$). In addition, with 25% PEG, more ungerminated seeds (10%) were recorded compared to those observed after soaking in 10% PEG ($P = 0.03$). Soaking in distilled water resulted in 12% more ungerminated seeds compared to those observed in 10% PEG ($P = 0.03$).

Unaged seeds soaked for 10 h:- The number of abnormal seedlings after soaking the seeds in 25% or 50% PEG increased by 9% or 15% respectively compared to the unaged control ($P < 0.02$). However, compared to the control there was a 23% or 17% increase in the number of abnormal seedlings when soaking occurred in water or 10% PEG respectively ($P < 0.01$). The number of abnormal seedlings obtained after

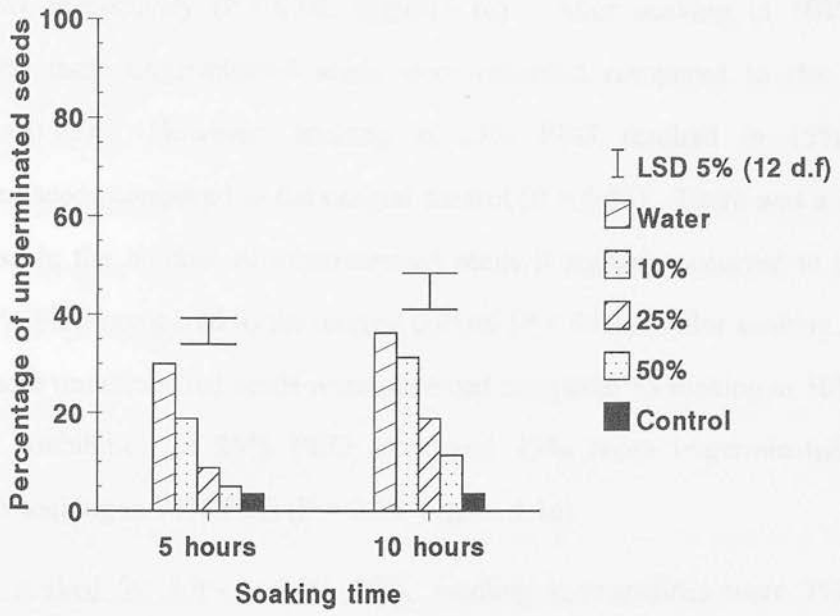


Figure 5.1e. Comparative effect of pre planting soaking in distilled water or polyethylene glycol (%) on the number of ungerminated seeds in unaged seeds of cultivar Epps.

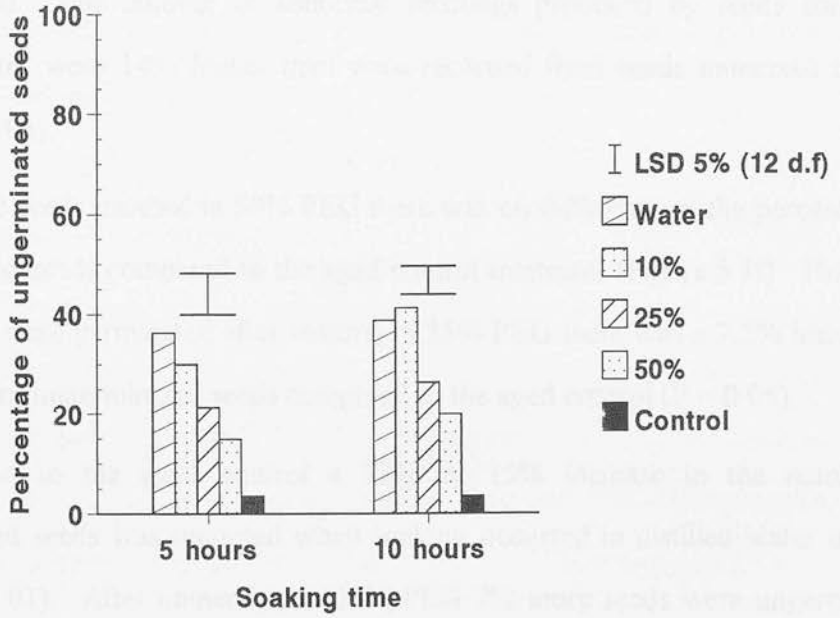


Figure 5.1f. Comparative effect of pre planting soaking in distilled water or polyethylene glycol (%) on the number of ungerminated seeds in aged seeds of cultivar Epps.

soaking in distilled water or 10% PEG was 15% or 10% higher compared to soaking in 25% PEG respectively ($P < 0.02$; Figure 5.1c). After soaking in 50% PEG, produced 8% more ungerminated seeds were recorded compared to the unaged control ($P = 0.03$). However, soaking in 25% PEG resulted in 15% more ungerminated seeds compared to the unaged control ($P = 0.03$). There was a 34% or 29% increase in the number of ungerminated seeds if soaking occurred in distilled water or 10% PEG compared to the unaged control ($P < 0.01$). After soaking in 25% PEG, 7% more ungerminated seeds were observed compared to soaking in 50% PEG ($P = 0.05$). Imbibition in 25% PEG, produced 13% more ungerminated seeds compared to soaking in 10% PEG ($P < 0.04$; Figure 5.1e).

Aged seeds soaked for 5 h:- In 25% PEG, seedling abnormalities were 7% lower compared to the aged control ($P < 0.05$). However, after soaking in 10% or 50% PEG the number of abnormal seedlings were similar to the aged control. Seeds soaked in distilled water showed 5% more abnormals compared to the aged control (Figure 5.1d). The number of abnormal seedlings produced by seeds soaked in distilled water were 14% higher than were recorded from seeds immersed in 25% PEG ($P < 0.03$).

For those seeds imbibed in 50% PEG there was no difference in the percentage of ungerminated seeds compared to the aged control treatment (Figure 5.1f). However, when seeds were germinated after soaking in 25% PEG there was a 7.5% increase in the number of ungerminated seeds compared to the aged control ($P = 0.05$).

Compared to the aged control a 22% or 15% increase in the number of ungerminated seeds was recorded when soaking occurred in distilled water or 10% PEG ($P < 0.01$). After immersion in 25% PEG 7% more seeds were ungerminated than recorded after soaking in 50% PEG ($P = 0.05$). After soaking in 25% PEG aged seeds produced 10% more ungerminated seeds compared to those produced after soaking in 10% PEG ($P = 0.02$). Soaking of the seeds in 10% PEG had 6% fewer

ungerminated seeds compared to soaking in distilled water ($P < 0.05$).

Aged seeds soaked for 10 h:- After imbibing the seeds in 10%, 25% or 50% PEG, there was no significant increase in seedling abnormalities compared to the aged control (Figure 5.1d). However, seeds soaked in distilled water showed a 27% increase in the number of abnormal seedlings compared to the aged control ($P < 0.01$). A quarter more seeds were abnormal after imbibition in water compared to that recorded after immersion in 10% PEG ($P < 0.01$).

It was observed that imbibition of aged seed for 5 or 10 h exacerbated the incident of seedling abnormalities particularly when soaking occurred in water or 10% PEG. In general seedling abnormalities increased with an increase in soaking time or decrease in PEG concentration. Seedling abnormalities were high in aged seeds compared to the unaged seeds ($P < 0.03$). Soaking of aged seeds for 5 h in 25% or 50% PEG maintained the control number of abnormal seedlings (Figure 5.1d).

After soaking in distilled water and in 10%, 25% or 50% PEG there was a 25%, 26%, 11% or 6% increase in the number of ungerminated seeds respectively compared to the aged control ($P < 0.02$; Figure 5.1f).

5.3.1.3. Days to 50% emergence

Unaged and aged seeds experienced delayed emergence if imbibed in distilled water compared to soaking in 25% polyethylene glycol, probably because soaking injury caused higher solute leakage (loss of membrane integrity) in distilled water (Figure 5.1g,h). Accelerated ageing, soaking time and PEG concentration alone produced a noticeable effect on the number of days to 50% emergence ($P > 0.03$).

Unaged seeds:- After 5 h imbibition in distilled water or the different concentrations of PEG the time to 50% emergence was delayed by 2.4 days compared to the control ($P < 0.03$). Seeds soaked for 5 h in distilled water or 10%, 25% and 50% PEG

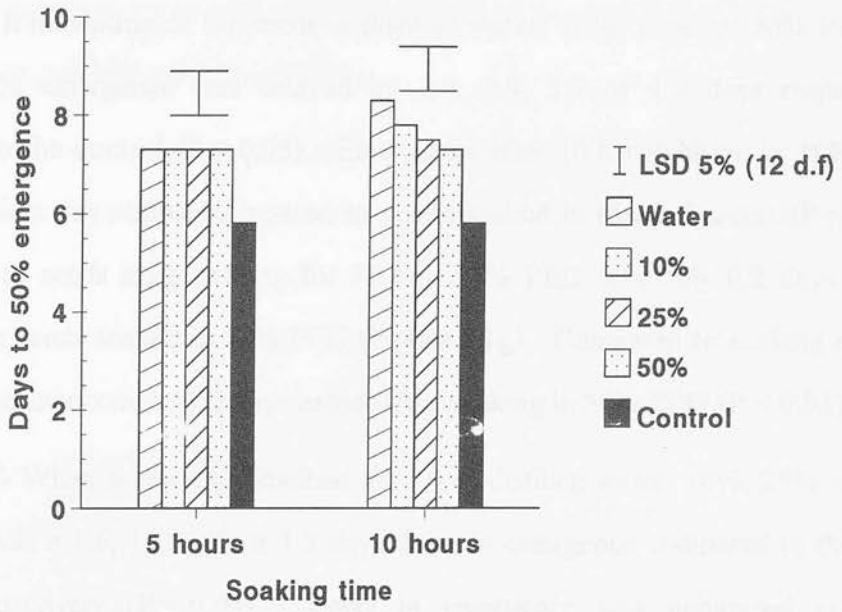


Figure 5.1g. Comparative effect of pre planting soaking in distilled water or polyethylene glycol (%) on days to 50% emergence in unaged seeds of cultivar Epps.

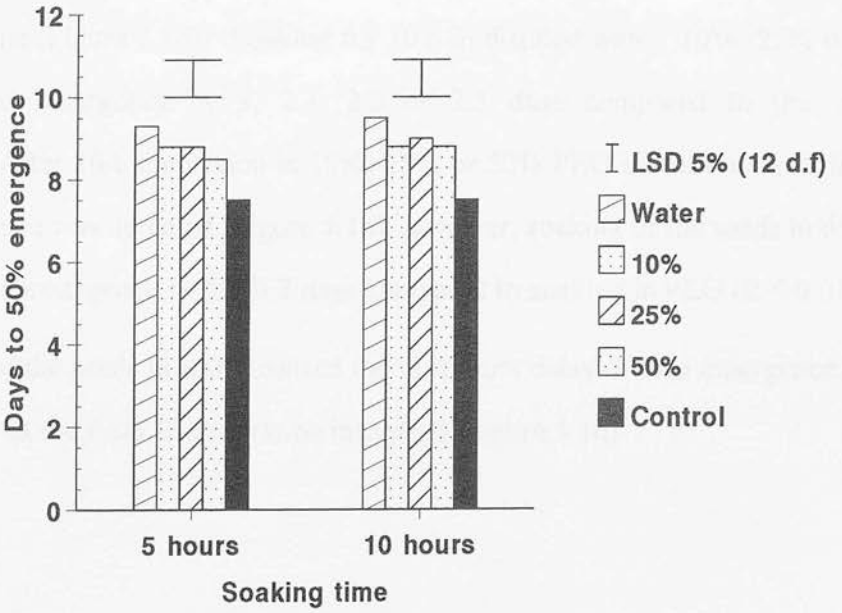


Figure 5.1h. Comparative effect of pre planting soaking in distilled water or polyethylene glycol (%) on days to 50% emergence in aged seeds of cultivar Epps.

completed 50% emergence on the same day (Figure 5.1g).

After 10 h imbibition of the seeds in distilled water, 10%, 25%, or 50% PEG the time to 50% emergence was delayed by 3.4, 2.9, 2.7 or 1.2 days respectively compared to the control ($P < 0.03$). Emergence after 10 h imbibition in 10% PEG occurred half a day sooner compared to seeds soaked in distilled water ($P < 0.01$). Emergence of seeds after soaking for 10 h in 25% PEG was only 0.2 days earlier compared to seeds soaked in 10% PEG (Figure 5.1g). Compared to soaking in 25% PEG, emergence occurred 1.5 days earlier after soaking in 50% PEG ($P < 0.01$).

Aged seeds:- When seeds were imbibed for 5 h in distilled water, 10%, 25% or 50% PEG there was a 1.6, 1.3, 1.2, or 1.2 days delay in emergence compared to the aged control respectively ($P < 0.04$). Delay in emergence was minimised as PEG concentration increased. Soaking in distilled water delayed emergence by 0.4 days compared to soaking in PEG ($P < 0.05$).

There were no difference in days to 50% emergence between the different PEG concentrations (Figure 5.1h). Soaking for 10 h in distilled water, 10%, 25% or 50% PEG delayed emergence by 3, 2.3, 2.5 or 2.3 days compared to the control ($P < 0.02$). After 10 h immersion in 10%, 25% or 50% PEG no difference in time to 50% emergence was detected (Figure 5.1h), however, soaking of the seeds in distilled water delayed emergence by 0.5-0.7 days compared to soaking in PEG ($P < 0.01$).

Soaking of the seeds in water caused the maximum delay in seed emergence. This was reduced as the PEG concentration increased (Figure 5.1h).

5.3.2. Experiment 5.2

Elevating soybean germination by pre-planting slow imbibition of unaged and aged seeds in polyethylene glycol (PEG).

Accelerated ageing reduced and delayed mean germination ($P < 0.02$). Unaged seeds pre-imbibed in water produced a similar number of normal seedlings as the controls, but the pre-imbibed seeds germinated 14 h earlier in a germination test conducted in rolled paper towel ($P < 0.01$). The results revealed that seeds subject to accelerated ageing showed a better response to pre-planting imbibition compared to the unaged seed sample. When low vigour seeds were imbibed in 25% polyethylene glycol instead of in water, germinability and growth were improved (Table 5.2).

5.3.2.1. Normal seedlings

There were no differences in the number of normal seedlings from the unaged seed lot subject to pre-planting imbibition in paper towels moistened with water or the various PEG solutions (Table 5.2). However, it was observed that from seeds subject to accelerated ageing the number of normal seedlings after pre-planting imbibition in distilled water was 12 to 20% lower compared to the seeds that were slowly imbibed in 10%, 25%, 35% or 50% PEG ($P < 0.03$).

Slow imbibition in 25% PEG produced the most normal seedlings when aged seeds were used (Table 5.2). The number of normal seedlings obtained after pre-planting slow imbibition of the aged seed sample in 10% or 50% PEG was 9% lower than those obtained after slow imbibition in 25% or 35% PEG ($P < 0.03$). Pre-planting slow imbibition in 25% PEG was the ideal treatment for maximum production of normal seedlings.

5.3.2.2. Abnormal seedlings and ungerminated seeds

Slow imbibition of unaged seeds in water or PEG before the germination test did not produce a difference in the number of abnormal seedlings or ungerminated seeds compared to the control seed sample (Table 5.2). However, seeds subject to accelerated ageing produced the least number of abnormal seedlings when imbibition occurred in 25% PEG ($P < 0.04$). The control seed sample and seed sample pre-imbibed in 50% PEG produced less abnormal seedlings than seeds pre-imbibed in water or 10% PEG. The number of ungerminated seeds decreased as the concentration of PEG increased. The control seeds produced most ungerminated seeds followed by those seeds imbibed in distilled water (Table 5.2). The number of ungerminated seeds after slow imbibition in 25% PEG was 10% higher than those obtained after imbibition in 35% or 50% PEG ($P = 0.03$).

5.3.2.3. Hours to 50% germination

Seeds subject to accelerated ageing took longer to complete 50% germination compared to control seeds ($P < 0.03$). The longer time to 50% germination in both unaged and aged seed samples was recorded for the control seed sample or seed sample imbibed in 50% PEG (Table 5.2). Seeds imbibed in distilled water germinated 11 h earlier compared to the control ($P < 0.01$). However, seeds imbibed in 10 or 25% PEG germinated at the same rate, but earlier than seeds imbibed in 35% PEG ($P < 0.04$). The number of days to 50% germination was inversely proportional to the osmotic concentration of PEG solution. Germination was not enhanced after imbibition in 50% PEG compared to the control (Table 5.2). Differences among pre-imbibed seeds in the number of hours to 50% germination between PEG concentrations were observed in both the unaged and aged seeds ($P < 0.03$).

Table 5.2. Mean data for normal seedlings, abnormal seedlings, ungerminated seeds and hours to 50% germination in unaged and aged seed samples as affected by the use of seeds that were pre-imbibed in various concentrations of polyethylene glycol (PEG) for 24 hours (mean of three replications).

Seed samples/PEG	Normal seedlings (%)	Abnormal seedlings (%)	Ungerminated seeds (%)	Hours to 50% germination
Unaged				
Control	98.7	1.3	0.0	48.0
Water	94.7	4.0	1.3	36.0
10%	97.3	1.3	1.3	42.0
25%	94.7	4.0	1.3	43.3
35%	93.3	4.0	2.7	46.0
50%	98.7	0.0	1.3	48.0
LSD at 5% (10 d.f)	6.8	5.1	3.2	1.7
Aged				
Control	58.7	25.3	16.0	56.0
Water	61.3	24.0	14.7	42.0
10%	68.0	22.7	9.3	49.0
25%	76.0	14.7	9.3	52.0
35%	72.0	20.0	8.0	56.0
50%	69.3	25.3	5.3	60.0
LSD at 5% (10 d.f)	7.3	7.1	5.2	1.3

5.3.3. Experiment 5.3

Minimising soaking injury by slow imbibition of high and low vigour soybean seeds in 25% polyethylene glycol (PEG) before germination.

Germinability was improved when the seeds were slowly imbibed in 25% PEG before the germination test. Improved germinability was particularly evident when aged seeds were used. Unaged seeds showed little response to osmoconditioning (Figure 5.3a-l).

5.3.3.1. Normal seedlings

Accelerated ageing decreased the number of normal seedlings in both the cultivars ($P < 0.03$). Initially Epps with large seeds produced 14% more normal seedlings compared to the small seeded cultivar Stafford ($P < 0.01$). Unaged seeds of both cultivars slowly imbibed in 25% PEG exhibited a similar number of normal seedlings to those imbibed in distilled water. However, the number of normal seedlings in aged seed lots were improved when seeds were slowly imbibed in 25% PEG (Figure 5.3a,b).

The number of normal seedlings in Epps declined by 21% after 2 days of accelerated ageing and by 46% after 4 days of accelerated ageing compared to the unaged seed lot ($P < 0.01$). However, Stafford exhibited 15% decline after 2 days of accelerated ageing and 50% decline after 4 days of accelerated ageing compared to the unaged seed lot ($P < 0.02$). Compared to the control slow imbibition in 25% PEG increased the number of normal seedlings by between 9% and 11% when aged seeds of large seeded cultivar Epps were used ($P < 0.03$). Whereas for Stafford the improvement was between 9% and 14% ($P < 0.02$).

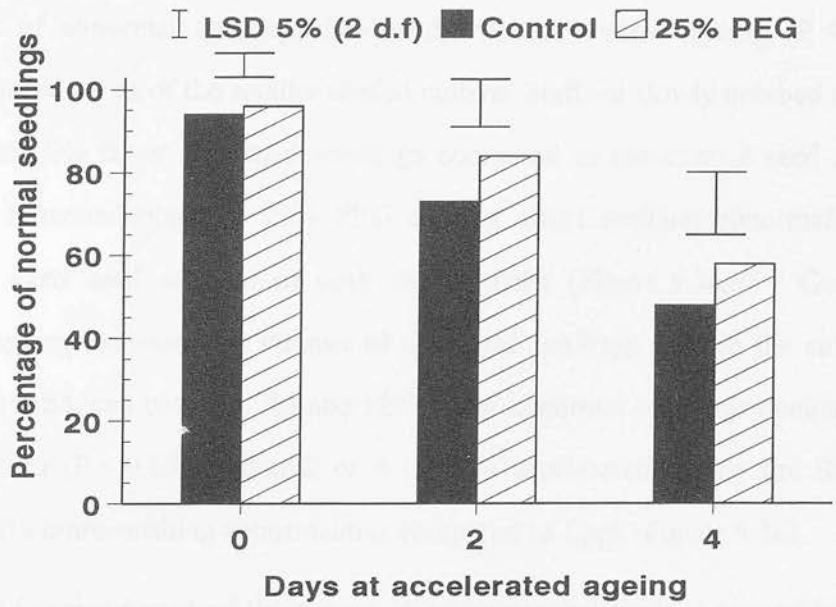


Figure 5.3a. Number of normal seedlings in ageing seeds of cultivar large seeded Epps germinated after 24 hours incubation in paper towels applied with distilled water or polyethylene glycol (PEG).

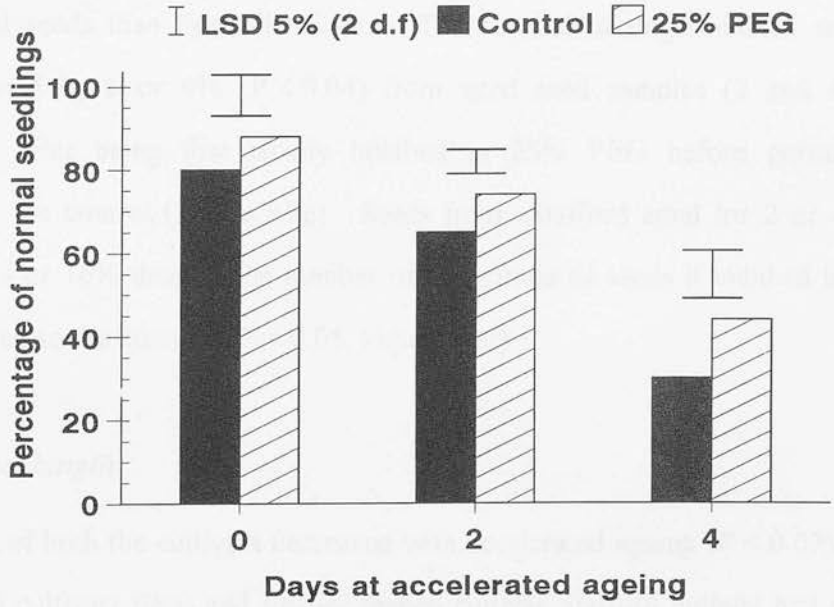


Figure 5.3b. Number of normal seedlings in ageing seeds of small seeded cultivar Stafford germinated after 24 hours incubation in paper towels applied with distilled water or polyethylene glycol (PEG).

5.3.3.2. Abnormal seedlings and ungerminated seeds

The number of abnormal seedlings increased with accelerated ageing ($P < 0.02$). However, unaged seeds of the smaller seeded cultivar Stafford slowly imbibed in 25% PEG produced 9% fewer abnormal seedlings compared to the control seed sample ($P < 0.04$). Slow imbibition in 25% PEG did not affect seedling abnormalities in unaged and aged seed samples of both the cultivars (Figure 5.3c,d). Generally accelerated ageing increased the number of abnormal seedlings in both the cultivars. Initially Epps produced between 7% and 12% fewer abnormal seedlings compared to cultivar Stafford ($P < 0.05$). After 2 or 4 days of accelerated ageing the Stafford produced 2-4% more seedling abnormalities compared to Epps (Figure 5.3c).

Accelerated ageing increased the number of ungerminated seeds in case of both the cultivars ($P < 0.02$). The unaged seed sample of Stafford produced 5% more ungerminated seeds than Epps (Figure 5.3e,f).

After being subject to accelerated ageing for 4 days Stafford produced 16% more ungerminated seeds than Epps ($P < 0.01$). The number of ungerminated seeds in Epps decreased by 6 or 9% ($P < 0.04$) from aged seed samples (2 and 4 days respectively) after being first slowly imbibed in 25% PEG before germination compared to the control (Figure 5.3e). Seeds from Stafford aged for 2 or 4 days showed a 5% or 16% drop in the number of ungerminated seeds if imbibed in 25% PEG compared to the controls ($P < 0.05$; Figure 5.3f).

5.3.3.3. Shoot length

Shoot length of both the cultivars decreased with accelerated ageing ($P < 0.03$). The larger seeded cultivars Epps and smaller seeded cultivar Stafford initially had similar shoot lengths. Shoot length of the unaged seed samples of both the cultivars imbibed in 25% PEG was similar to that attained by control seed sample (Figure 5.3g,h).

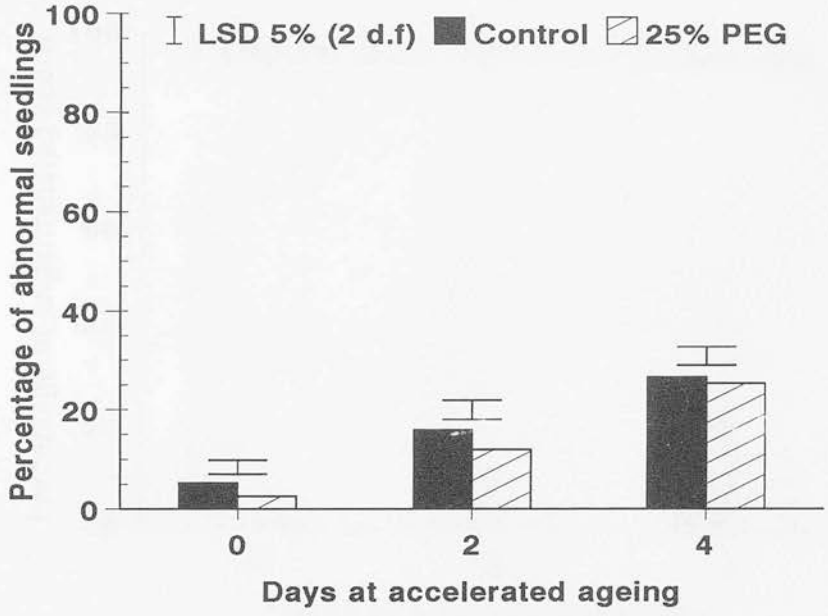


Figure 5.3c. Number of abnormal seedlings in ageing seeds of large seeded cultivar Epps germinated after 24 hours incubation in paper towels applied with distilled water or polyethylene glycol (PEG).

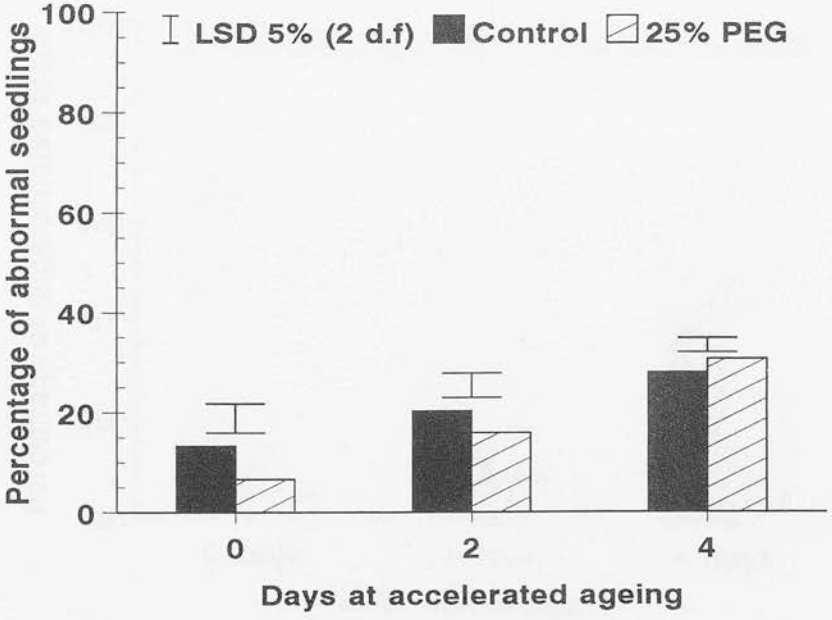


Figure 5.3d. Number of abnormal seedlings in ageing seeds of small seeded cultivar Stafford germinated after 24 hours incubation in paper towels applied with distilled water or polyethylene glycol (PEG).

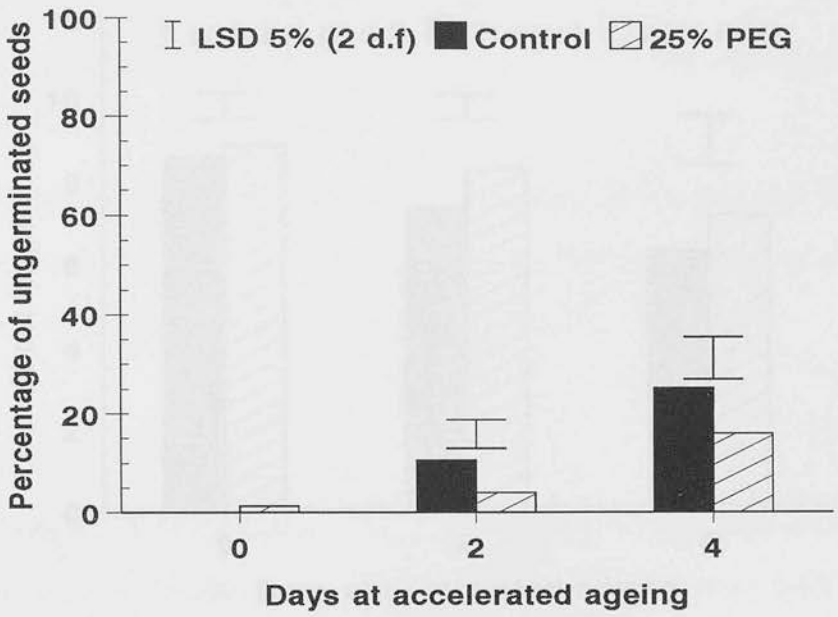


Figure 5.3e. Number of ungerminated seeds in ageing seeds of large seeded cultivar Epps germinated after 24 hours incubation in paper towels applied with distilled water or polyethylene glycol (PEG).

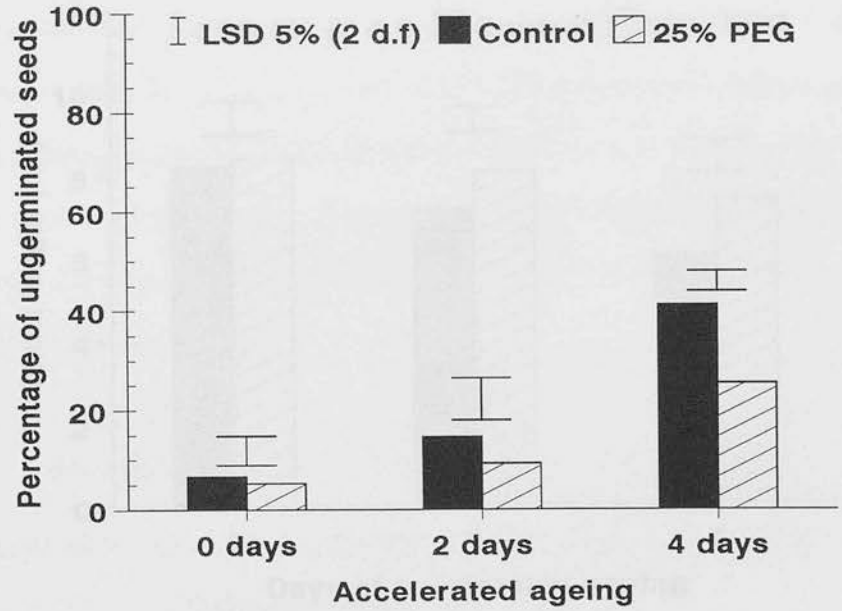


Figure 5.3f. Number of ungerminated seeds in ageing seeds of small seeded cultivar Stafford germinated after 24 hours incubation in paper towels applied with distilled water or polyethylene glycol (PEG).

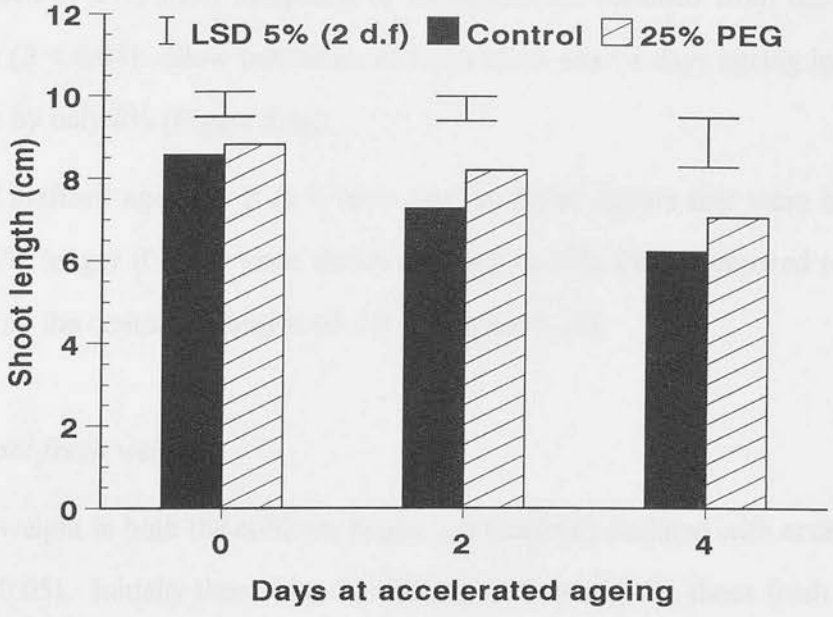


Figure 5.3g. Shoot length from ageing seeds of large seeded cultivar Epps germinated after 24 hours incubation in paper towels applied with distilled water or polyethylene glycol (PEG).

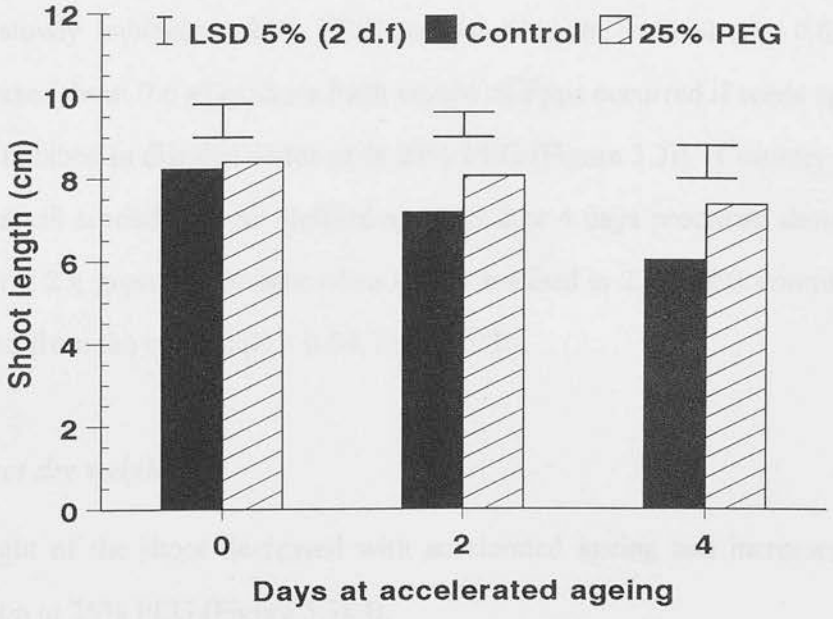


Figure 5.3h. Shoot length from ageing seeds of small seeded cultivar Stafford germinated after 24 hours incubation in paper towels applied with distilled water or polyethylene glycol (PEG).

For Epps aged for 2 days a 15% increase in shoot length was recorded from seeds slowly imbibed in 25% PEG compared to measurements obtained from the control seed sample ($P < 0.04$). Slow imbibition of Epps seeds after 4 days ageing increased shoot length by only 8% (Figure 5.3g).

Seeds of Stafford aged for 2 or 4 days also produced shoots that were between 10% and 15% longer if seeds were slowly imbibed in 25% PEG compared to those produced from the control treatment ($P < 0.02$; Figure 5.3h).

5.3.3.4. Shoot fresh weight

Shoot fresh weight in both the cultivars (Epps and Stafford) declined with accelerated ageing ($P < 0.05$). Initially there were no differences between the shoot fresh weight for both cultivars. For unaged seed of both cultivars shoot fresh weight was similar even when seeds had been slowly imbibed in 25% PEG (Figure 5.3i,j).

In Epps aged for 2 days, however, shoot fresh weight increased by 0.9 g when the seeds were slowly imbibed in 25% PEG compared to the control ($P < 0.05$). A smaller increase (about 0.6 g) in shoot fresh weight of Epps occurred if seeds aged for 4 days were imbibed in distilled water or in 25% PEG (Figure 5.3i). Contrary to this seeds of the small seeded cultivar Stafford aged for 2 or 4 days produced shoots that were 1.5 g or 1.2 g greater in weight when slowly imbibed in 25% PEG compared to those obtained from the control ($P < 0.04$; Figure 5.3j).

5.3.3.5. Shoot dry weight

The dry weight of the shoot decreased with accelerated ageing and increased with slow imbibition in 25% PEG (Figure 5.3k,l).

Slow imbibition had no effect on shoot dry weight of unaged seeds of Epps or Stafford. Epps aged for 2 and 4 days produced an increase (about 0.2 g) in the dry

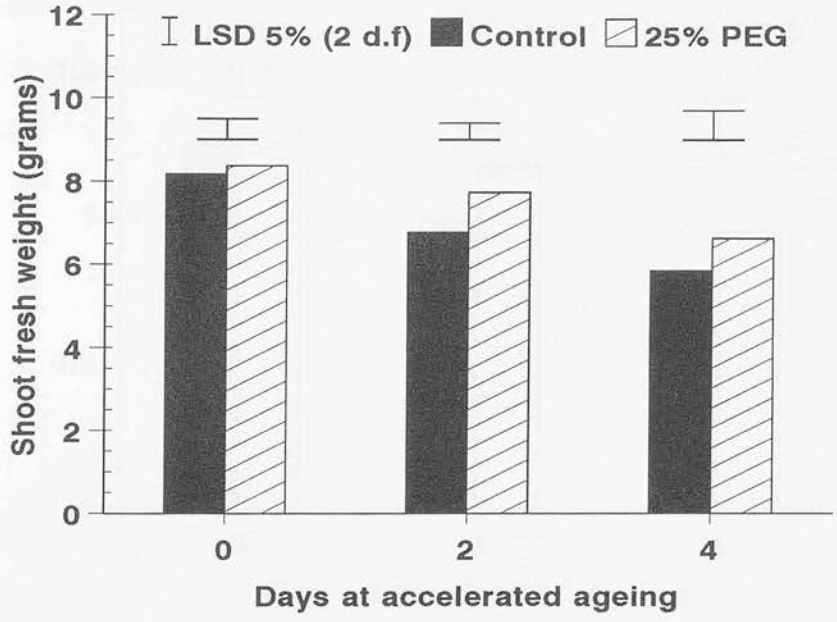


Figure 5.3i. Shoot fresh weight from ageing seeds of large seeded cultivar Epps germinated after 24 hours incubation in paper towels applied with distilled water or polyethylene glycol (PEG).

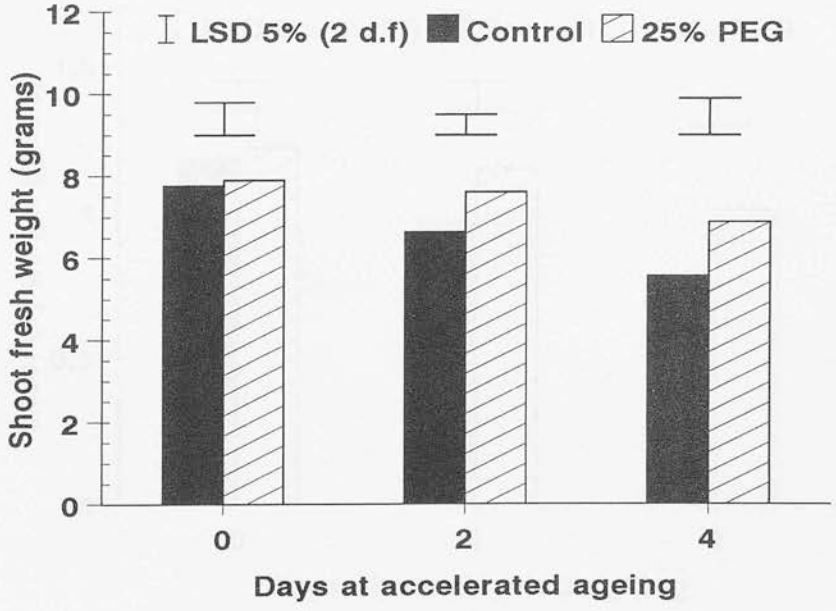


Figure 5.3j. Shoot fresh weight from ageing seeds of small seeded cultivar Stafford germinated after 24 hours incubation in paper towels applied with distilled water or polyethylene glycol (PEG).

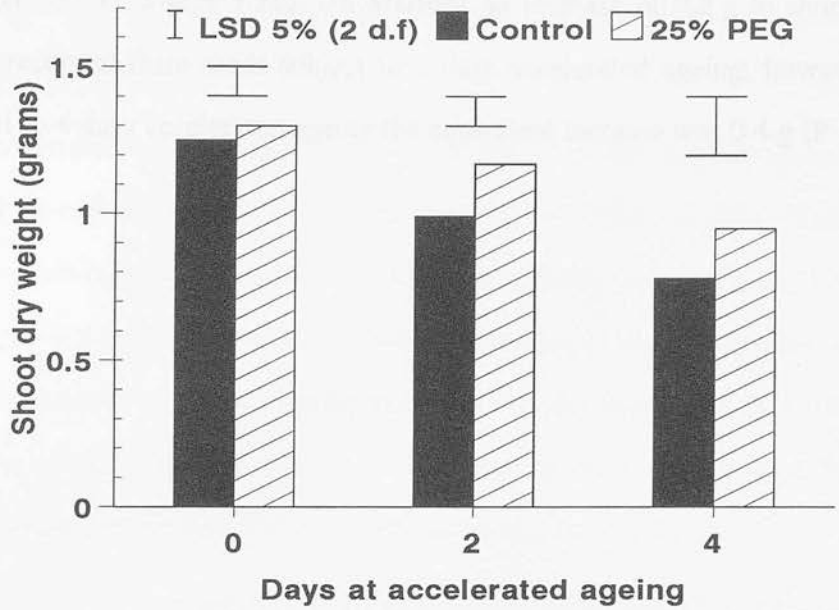


Figure 5.3k. Shoot dry weight from ageing seeds of large seeded cultivar Epps germinated after 24 hours incubation in paper towels applied with distilled water or polyethylene glycol (PEG).

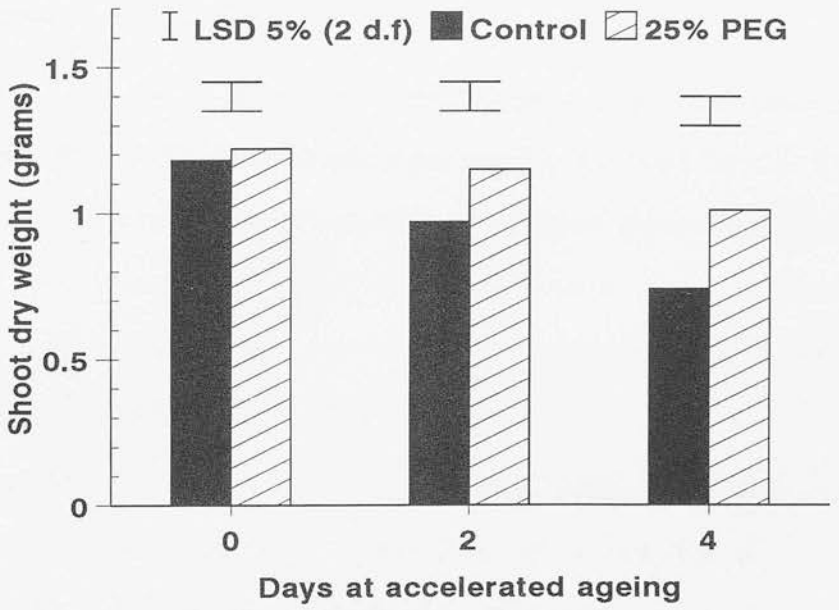


Figure 5.3l. Shoot dry weight from ageing seeds of small seeded cultivar Stafford germinated after 24 hours incubation in paper towels applied with distilled water or polyethylene glycol (PEG).

weight of the shoot when the seeds were slowly imbibed in 25% PEG compared to the control ($P < 0.03$; Figure 5.3k). In Stafford an increase of 0.2 g in shoot fresh weight was recorded from seeds subject to 2 days accelerated ageing, however, for seeds subject to 4 days accelerated ageing the equivalent increase was 0.4 g ($P < 0.03$; Figure 5.3l).

5.3.4. Experiment 5.4

Effect of osmotic stress applied through different concentrations of polyethylene glycol (PEG) on germinability of unaged and aged seeds.

For both the unaged and aged seed samples the ability of the seeds to germinate in rolled paper towels relied upon the concentration of PEG solution (Table 5.4). Seedling fresh weight decreased with an increase in PEG concentration, however, exceptionally, more number of seeds germinated in the paper towels supplied with 5% or 10% PEG compared to paper towels supplied with distilled water ($P < 0.01$). In paper towels supplied with 5 or 10% PEG seedling fresh weight was lowered compared to paper towels supplied with distilled water (Table 5.4).

The unaged seed sample exhibited 16% more seedlings compared to the aged seed sample ($P = 0.01$). Seedling fresh weight of the unaged seed sample was 13% higher than the aged seed sample ($P = 0.02$).

5.3.4.1. Germination percentage

When unaged seeds were subject to a germination test between paper towels moistened with 5% or 10% PEG, seedling germination decreased by only 3 to 4% respectively compared to seeds germinated in paper towels moistened with distilled water (Table 5.4). However, compared to the distilled water treatment, there was a 13%, 22% or 33% reduction in germination for seeds subject to treatment with 15%, 20% or 25% PEG respectively ($P < 0.02$).

Aged seeds produced the lowest germination when distilled water was used, but germination was 11% higher in paper towels supplied with 5% or 10% PEG ($P < 0.02$). When aged seeds were germinated in paper towels to which 15% or 20% PEG had been applied the number of seedlings were 6% higher than those obtained in paper towels supplied with distilled water, but 5% less compared to those obtained

when the paper towels were supplied with 5% or 10% PEG (Table 5.4).

Aged seeds experienced a large (45%) drop in the number of germinated seeds when germination occurred in paper towels applied with 25% PEG ($P < 0.01$). When germinated in paper towels wetted with water, aged and unaged seeds showed 32% less germination than those wetted with 25% PEG ($P = 0.01$). In addition, aged seed produced 18%, 15%, 13%, or 29% less seedlings when paper towels were supplied with 5%, 10%, 15%, or 25% PEG respectively ($P < 0.03$; Table 5.4).

5.3.4.2. Seedling fresh weight

Fresh weight of seedlings decreased by 21%, 28%, 41%, 60% or 69% when unaged seeds were germinated in paper towels supplied with 5%, 10%, 15%, 20% or 25% PEG respectively compared to the unaged controls ($P < 0.01$). Aged seeds experienced a 28%, 37%, 50%, 61% or 81% decline in the fresh weight of the seedlings in 5%, 10%, 15%, 20% or 25% PEG respectively compared to the aged seeds germinated in rolled paper towel wetted with distilled water (Table 5.4).

Aged and unaged seeds followed similar behaviour in that fresh weight of the seedlings was reduced as PEG concentration increased. However, the decline in the fresh weight of seedlings at each level of PEG was more rapid in case of aged seeds (Table 5.4).

5.3.4.3. Moisture uptake

Generally, seeds subject to accelerated ageing absorbed more moisture compared to unaged seed sample (Table 54). Unaged seeds absorbed 8%, 19%, 23%, 28% or 35% less moisture when imbibition occurred in paper towels supplied with 5%, 10%, 15%, 20% or 25% PEG respectively compared to absorption that took place in paper towels wetted with distilled water ($P < 0.04$). Aged seeds also absorbed 9%, 21%,

Table 5.4. Mean data for germination percentage, ungerminated seeds and seedling fresh weight in unaged and aged seed samples germinated in paper towels applied with various concentrations of polyethylene glycol (PEG). Percentage moisture reached was measured after 24 hours imbibition in paper towels applied with various concentrations of PEG (mean of four replications).

Seed samples/PEG	Germination percentage (%)	Ungerminated seeds (%)	Seedling fresh weight (g)	Moisture content reached (%)
Unaged				
Control (water)	92.0	8.0	9.13	60.3
5%	89.0	11.0	7.13	52.2
10%	88.0	12.0	6.50	43.0
15%	79.0	21.0	5.38	38.5
20%	70.0	30.0	3.63	32.0
25%	59.0	41.0	2.75	25.5
LSD at 5% (10 d.f)	5.4	5.5	1.2	16.9
Grand mean	79.5	20.5	5.75	41.9
Aged				
Control (water)	60	40.0	8.00	70.7
5%	71	29.0	5.75	61.6
10%	72.0	28.0	5.00	50.3
15%	66.0	34.0	4.00	44.0
20%	64.5	35.5	3.13	35.5
25%	30.0	70.0	1.50	27.3
LSD at 5% (10 d.f)	6.0	6.1	0.9	18.9
Grand mean	60.6	39.4	4.56	48.2

27%, 36% or 43% less moisture when imbibition occurred in 5%, 10%, 15%, 20% or 25% PEG instead of distilled water ($P < 0.02$).

5.4. DISCUSSION

Experiment 5.1 studied the relationship of germinability to the rate of imbibition in unaged or aged seeds. It was observed that the rate of water uptake in case of both high or low vigour seeds was reduced if seeds were imbibed in 25% PEG rather than in distilled water. The data indicate that it is possible to prevent the injury that normally occurs when aged seeds are imbibed in water.

Tilden & West (1985) reported the reversal of the effect of ageing by slowing down the rate of water uptake in low vigour soybean seeds. In experiment 4.1 failure of the seeds especially those of low initial vigour to show normal and speedy germination if imbibed in distilled water can be associated with high solute leakage. These findings support the conclusions made by Roos (1984a). Lack of oxygen (anoxia) may have played some role as seeds were not expected to suffer from soaking injury after soaking in 25 or 50% PEG. Further reduction in the number of normal seedlings after 10 h soaking compared to 5 hours soaking increased the chances of loss in normal germinability to be partly due to anoxia. Since O_2 (oxygen) concentration in 25% or 50% PEG is probably lower than in water, seeds soaked in these solutions may have been more likely to suffer due to anoxia and that is perhaps why seeds soaked in 10% or 25% had reduced germination compared to the control seed lot.

The harmful effects of soaking soybean seeds in water might be due to loss of membrane integrity, loss of essential nutrients and increase in the activity of bacteria and fungi attracted and stimulated by seed exudates. However, according to Roos & Pollock (1971) while the seeds are submerged in water cellular death due to anoxia can also be the cause of loss in seed vigour and viability.

Many research studies have indicated that loss of vigour in seeds may be related to changes in cellular membranes (Abdul-Baki, 1969). Others have interpreted increased

leakage of electrolytes and a decline in respiratory activity during accelerated ageing as a possible consequence of membrane deterioration in soybean (Parrish & Leopold, 1978)

Grable & Danielson (1965) found that pathogens developed rapidly on germinating seeds and roots under saturated soil conditions. However, these results (experiment 5.1) showed that soaking in PEG minimised soaking injury and resulted in improved germination compared to seeds germinated after soaking in distilled water.

This indicated that if the seed is not dormant and is genetically capable of normal germination then soaking injury may result in the failure of seed membranes to perform normal functions, particularly in aged seeds. Therefore, soaking injury due to excess water can be regarded as the most likely cause of germination failure under certain conditions. The possible cause of germination failure is expected to be membrane disruption. In other words germination failure or tendency towards seedling abnormalities seem not to be a result of failure of the seeds to synthesise food materials, but due to a disruption of the cell membranes to organise biochemical events during germination. These ideas have been supported by the results of Schoettle & Leopold (1984).

There is no final proof, but it can be suggested that the causes of failure in germination and tendency towards abnormalities vary from seed to seed and with germination conditions. Since it has been well established that ruptured seed coats in soybean were caused by the cotyledons expanding against the seed coat (Hill *et al.*, 1986a). Due to excess water causing rapid absorption in aged seeds soaked in distilled water (experiment 5.1) expansion of the cotyledons against the seed coat may have ruptured the seed coat as aged soybean seeds are reported to absorb more water and at a higher rate compared to unaged seeds thus encouraging greater seed coat or cellular rupture that lead to poor germinability (Parrish and Leopold, 1977; Hill *et al.*, 1986a).

Woodstock & Taoi (1981) controlled imbibition injury by slowly imbibing the embryonic axis of soybean seeds in PEG and suggested this possibility in peas. The effectiveness of 25% PEG in controlling soaking injury might be due to osmotic reduction in the rate of water uptake. Water uptake injury and its reversal with PEG have been reported by Powell & Matthews (1978) in peas and by Woodstock & Taoi (1981) in soybean.

Previous workers have confirmed that submerged unaged and aged seeds produced much higher levels of ethanol and acetaldehyde compared to seeds that were directly germinated on moist paper towels (Woodstock & Taylorson, 1981). Higher levels of ethanol and acetaldehyde production could possibly be the cause of soaking injury and germination failure in case of the seeds imbibed in distilled water (experiment 5.1). The rate of the production of ethanol and acetaldehyde may be directly proportional to the rate of water absorption. However, further research is needed to verify this phenomenon.

These results also indicated that germinability, particularly in case of low vigour seed samples can be improved if the seeds are slowly imbibed in 25% PEG compared to distilled water. In addition seeds imbibing in PEG emerged earlier compared to seeds soaked in distilled water. This suggested that submergence of the seeds in distilled water for 5 or 10 h probably allow loss of sufficient organic solutes to cause a delay in emergence. Aged seeds soaked in PEG also delayed emergence, but not by as much as caused by submergence in distilled water. This behaviour of the seeds in the present experiment has provided an interesting relationship between seed ageing and solute leakage, but further experimentation is needed to find out, if extensive solute leakage can be related to delayed emergence especially in aged seeds.

Accelerated ageing and solute leakage were both responsible for delay in emergence. Delay in emergence was, however, minimised with an increase in the PEG concentration of imbibing solution. Simon & Raja-Harun (1972) arrived at

similar conclusions. This means that solute leakage can be used as a possible indicator of comparative storability in different soybean cultivars. Temporary water logging is a common feature of tropical and subtropical cropping systems. However, some research workers have reported that after successful emergence, soybean, compared to other legumes, is tolerant to short term water logging, and if not attacked by pathogens, recovers, quickly after the water logging is over (Stanley *et al.*, 1980).

To obtain maximum emergence under saturated tropical and subtropical conditions is a priority. Seeds may need to be pre-conditioned to attain successful emergence if they are subject to excess moisture. However, the current results have indicated that saturated soil conditions should be avoided if satisfactory crop stands are to be expected.

The number of normal seedlings produced by aged seed sample was increased when the seeds were germinated after slow imbibition for 24 hours in increasing PEG concentration (experiment 5.2). In aged seeds maximum number of normal seedlings were produced if slow imbibition occurred in 25% PEG. These findings further confirmed the findings of Mukherji & Dey (1985) who obtained improved germination from pre-conditioned soybean seeds. Therefore, the effects of accelerated ageing can be partially reversed by slowly imbibing the seeds in 25% polyethylene glycol. This practice probably allows the seed membranes of aged seeds to absorb water slowly which otherwise would be damaged by water uptake. Reversal of the effects of accelerated ageing and soaking injury in soybean have previously been reported by Oliveira *et al.* (1984) and Woodstock & Taylorson (1981) respectively. These results further confirmed the widely accepted idea that pre-hydrated seeds show improved and enhanced germination compared to non pre-hydrated seeds. This behaviour of pre-hydrated seeds has also been reported by Ikeda (1985) and Schultz & Evenson (1983). Experiment 5.2 studied the relationship of

pre-hydrated seeds to the number of normal seedlings, seedling abnormalities as well as the number of ungerminated seeds. The effect of slow imbibition, however, varied with the seed samples. Unaged seeds pre-imbibed in water produced a similar number of normal seedlings as was produced by seeds pre-imbibed in PEG. However, the unaged seeds germinated 8 h sooner. The low vigour seeds pre-imbibed in water showed early germination, but produced a lower number of normal seedlings compared to the seeds imbibed in PEG. This means that if low vigour seeds were used and quick germination is not a priority then slow imbibition in 25% PEG is ideal for improved germination. However, if high vigour seeds were used then pre-imbibition in 25% PEG or distilled water produced similar results (experiment 5.2). Therefore, such seeds need only to be pre-imbibed in paper towels moistened with water to achieve quicker germination. In the tropical and subtropical regions where according to Emerson (1982) high soil temperatures have been reported to be a primary contributing factor to poor stand establishment, the practice of planting pre-hydrated seeds may be beneficial particularly if sowing is carried out later in the day. This will reduce the risk of exposing the seeds to high temperatures in the afternoon, allow enough time and favourable conditions for seed-soil contact, avoid moisture stress and enhance germination. Previous findings agree with the findings of this experiment (Andrews, 1982; Dadson, 1982).

It is very important to ensure that planting seed is viable and capable of vigorous growth. However, viable and vigorous seeds do not ensure planting success unless planted in favourable germination conditions. Soybean can be successfully grown in most of the third world countries, but successful emergence of the seedlings from the soil is a problem. In most cases even high vigour seeds give poor crop stands (Dadson, 1982).

Parrish and Leopold (1977) suggested that membrane injury is critical only in the first few minutes of imbibition, however, in experiment 5.2, 24 h imbibition treatment

in 25% PEG probably allowed enough time for repair and reorganisation of the membranes especially in case of aged seeds. Alongside, a sensible selection of varieties, raising of soybean seeds to a certain pre-determined imbibition stages need to be tested in the field.

In experiment 5.3 slow imbibition in PEG improved seed viability and seedling vigour compared to seeds that were imbibed in distilled water. The idea that low vigour soybean seeds are more vulnerable to imbibition damage compared to high vigour seeds had been earlier established (Parrish *et al.*, 1982; Halmer & Bewley, 1984). Unlike high vigour seeds the weakly living membranes of the low vigour seeds may have lost their ability to cope with rapid imbibition and subsequent germination particularly when the initial rate of water absorption was high (experiment 5.3). This initial inrush of water is responsible for a major part of soaking injury in all legumes (Kidd & West, 1918) and particularly in the case of low vigour seeds of the modern soybean cultivars (Hunter & Erickson, 1952).

The results indicated that the initial inrush of water into the low vigour seeds was probably responsible for a noticeable drop in the percentage of normal seedlings, and may also have caused a significant reduction in shoot length and shoot fresh or dry weight. All these parameters showed a notable increase when the rate of water uptake was controlled by imbibing the seeds in 25% PEG.

Das *et al.* (1989) found that after pre-planting soaking in PEG rice (*Oryza sativa*) seeds had an increased plant height, tiller number and shoot dry weight. The results also indicated that the rate of water absorption in the initial 24 h of imbibition improved seedling vigour and that this was reflected through an increase in shoot weight and shoot length when the rate of moisture uptake was controlled by allowing the seeds to imbibe slowly in 25% PEG.

Soaking injury, if extensive, inflicts severe damage on germinability. For similar

reasons Ragus (1987) suggested that selection of those soybean cultivars that are less affected by soaking injury is the first step to successful germination under saturated soil moisture conditions. This type of situation is very common in the tropics and subtropics (Troedson *et al.*, 1983). It was observed that the low vigour seed samples of both the cultivars were particularly vulnerable to damage from rapid water uptake and responsive to slow imbibition in 25% PEG. It is suggested that partly imbibed seeds may increase the chances of getting satisfactory crop stands under field conditions especially if seed vigour is low.

In experiment 5.4, unaged seeds did not show a decrease in the number of germinated seeds because they were not affected by soaking injury in water. This indicates that the amount of soaking injury is related to the vigour of the seeds. Unaged seeds could withstand soaking injury to some extent. Slow germination in paper towels supplied with more than 15% PEG delayed and even prevented germination of some seeds. Unaged seeds have been reported to show comparative resistance to soaking injury compared to low vigour seeds (Abdul-Baki & Anderson, 1973). The results also showed that compared to water, lower vigour seeds showed improved germination in paper towels supplied with 5% or 10% PEG. However, the increase in germination percentage in paper towels supplied with 5% or 10% PEG was not accompanied by an increase in the fresh weight of the seedlings. Instead seedling fresh weight in case of paper towels supplied with 5 or 10% PEG was drastically reduced. Increase in germinated seeds and decrease in seedling fresh weight in case of aged seeds may be due to the ability of low vigour seeds to avoid soaking injury in 5% or 10% PEG, but their inability to maintain subsequent speedy growth due to osmotic inhibition. The high moisture absorption rate of the lower vigour seeds in distilled water indicated that soaking injury could result.

Summer planting of soybean in Pakistan is subject to stressful conditions (high soil temperature & lower soil moisture). After sowing moisture evaporation is high. The

soil becomes drier and drier and the seeds suffer badly from reverse osmosis. The above experiment has proved that moisture stress not only effect germination percentage, but also has a substantial decreasing effect on seedling fresh weight.

It is believed that slow imbibition in PEG enables those seeds to produce a normal seedling, that may not exhibit normal germination if the rate of water uptake is high. Moreover, slow imbibition in PEG enable those seeds to produce an abnormal seedling, that may fail to germinate under rapid rate of water uptake.

It is concluded that reduction in the rate of water uptake increase normal seedlings at the expense of seedling abnormalities together with an increase in seedling abnormalities at the expense of preventing the number of ungerminated seeds.

Reducing the rate of imbibition is not likely to improve germination of those seeds that are potential abnormal or potential ungerminated (on genetical and biochemical level etc.) and will not respond to the best of germination conditions including an ideal rate of water uptake.

CHAPTER 6

EVALUATION OF VARIETIES FOR STORAGE POTENTIAL AND RESISTANCE TO SOAKING INJURY

6.1. INTRODUCTION

A germination test after accelerated ageing has widely been used as a criterion of storability in soybean, but lack of standardisation has resulted in a variation in results. This is due to the great difference in equipment and conditions from laboratory to laboratory.

Byrd & Delouche (1971) found that accelerated ageing of soybean seeds at 42°C and 100% relative humidity for 48 h followed by a germination test distinguished seed samples according to their ability to survive. However, Wilcox *et al.* (1975) reported that fungal growth on seeds was a problem with the standard procedure. Moreover, it was reported that accelerated ageing of the seeds at 40°C and 75% relative humidity for between 1 and 3 weeks enabled the selection of lines that possessed good storage characters.

Parrish & Leopold (1978) reported that physiological changes in seeds that are subjected to accelerated ageing are similar to those reported under natural conditions. Hinson & Hartwig (1982) suggested that soybean cultivars with high quality seeds that have the potential to retain their high seed vigour and viability during storage should be recommended for tropical and subtropical regions. Soybean seed characteristics that are associated with good storability are also associated with resistance to solute leakage. For example small seed size (Vanangamudi, 1988) hardseededness (Potts *et al.*, 1978) and black seed coat colour (Maryushkin *et al.*,

1987) are reported to be associated with both good storage life and resistance to water uptake injury.

Seeds with large size (Carlton & Cooper, 1972) and light seed colour (Koslanund & Delouche, 1987) produced enhanced germination, but were susceptible to soaking injury. It is known that cell constituents of deteriorated seeds leak more solutes than non deteriorated seeds. The amount of solute leakage is frequently related to the degree of seed deterioration.

It is only after emergence, that a seedling can interact with the above ground environment to produce a satisfactory crop. High emergence and acceptable crop stand are essential to profitable soybean husbandry.

Soybean cultivars with a better storage potential, resistance to imbibition damage and vigorous growth are some of the priorities to be considered before screening varieties for tropical and subtropical regions where obtaining consistently good plant stand is a serious problem. In tropics and subtropics rapid seed deterioration is common due to unfavourable preharvest and postharvest environmental conditions. Kueneman (1982) had similar views about soybean planting in the tropics and subtropics.

In tropical and subtropical countries, conditions such as high soil temperatures and crust formation before emergence together with poor quality seeds contribute to poor stand establishment (Emerson, 1982).

Moreover, some soybean varieties are resistant to stressful planting conditions than others. If moisture stress is a threat then soybean cultivars with small seeds are suitable because they germinate and emerge quickly (Dadson, 1982). However, research regarding the superiority of cultivars with small seeds over cultivars with larger seeds has been conflicting.

Edward & Hartwig (1971) reported that the smaller or medium sized soybean

seeds were superior in rate of emergence and root development. However, forage legume cultivars with larger seeds exhibited a greater overall emergence percentage and yielded more than a variety with smaller seeds (Carlton & Cooper, 1972).

The above literature suggested that investigation of various soybean cultivars for storage potential and resistance to imbibition damage and to identification of seed characteristics responsible for these traits may be useful.

6.2. SPECIFIC METHODS

6.2.1. Experiment 6.1

Relationship of accelerated ageing and soaking in distilled water in six soybean cultivars.

The cultivars studied were small seeded Essex and medium seeded Stonewell (American), large seeded Opale and Gemma (French) and small seeded Pb-1 and Kalitur (Indian).

Seeds were either subject to accelerated ageing for 4 days in a desiccator placed in a water bath at 41°C (between 95% and 100% relative humidity) or soaked in distilled water for 12 h at 25°C. A separate batch of untreated seeds of each variety were kept as controls. Seeds were treated for 5 seconds in 1% sodium hypochlorite.

Germination tests were conducted at 25°C, in 2.5 cm deep in sand in a growth room (12 h/d photoperiod). Aged and unaged seeds were germinated 12 h earlier to equate to seeds that had been soaked for 12 h before germination. Data on the number of normal seedlings, number of abnormal seedlings and hypocotyl length were recorded 12 days after planting. An estimate of ungerminated seeds was also made. Details in chapter 3.

6.2.2. Experiment 6.2

Evaluation of eight soybean cultivars having different seed characteristics.

Seeds of cultivars Cumberland, Woodworth, Epps, Pixie, 79-W-220, Swat-84, Weber and 80-B-4007 from the summer (kharif) 1991 crops were supplied by the North West Frontier Province Agricultural University Research Station, Mingora, Pakistan.

All seeds were kept at room temperature for 4 days to equilibrate with the surrounding relative humidity. Approximately 100 g of seed of each cultivar were subjected to accelerated ageing for 4 days as in experiment 6.1. Cultivars were

isolated in muslin bags inside the desiccator. Another 100 g of each cultivar was used as unaged control. After accelerated ageing the seeds were allowed to dry at room temperature for 2 days.

Seeds were treated for 5 seconds in 1% sodium hypochlorite and then planted 2.5 cm deep in the growth room in plastic trays containing compost (25°C; 12 h/d photoperiod). Data were recorded after 12 days on the number of normal seedlings, number of abnormal seedlings and number of ungerminated seeds and shoot length. The fresh and dry weights of the shoot were also determined (grams). Details are given in chapter 3.

6.2.3. Experiment 6.3

Comparative resistance of nine soybean cultivars to accelerated ageing and soaking injury.

Seeds of the cultivars Emerald, Ripley, Morgan, Kent, York, Conrad, Stafford, Linford, and Manokin were subjected to accelerated ageing for 3 days as in experiment 6.1.

After accelerated ageing higher seed moisture contents were attained by Emerald, Ripley, Morgan, Kent, York, Conrad, Stafford, Linford, and Manokin. A separate batch of seeds of each cultivar not subject to accelerated ageing was used as a control seed sample. The seeds were allowed to dry at room temperature for 4 days.

The seeds were treated and germinated as in experiment 6.2. Data were recorded after 12 days on the number of normal seedlings, number of abnormal seedlings and number of ungerminated seeds. The fresh weight of the shoot was also recorded (grams). Seeds were soaked in high purity water and the conductivity (μSg^{-1}) of the soak solution was recorded after 30 minutes or after 4 h. Details are given in chapter 3.

6.3. RESULTS

6.3.1. Experiment 6.1

Relationship of accelerated ageing and soaking in distilled water in six soybean cultivars.

The moisture content of the seeds increased after accelerated ageing; Essex (from 8.7 to 28.6%), Stonewell (from 9.3 to 28.7%), Opale (from 10.9 to 30.5%), Gemma (from 11 to 32.1%), Pb-1 (from 8.4 to 27.6%) and Kalitur (from 11.3 to 26.9%).

After accelerated ageing the cultivars showed marked differences in the rate of loss of germinability. Cultivars that exhibited a rapid fall in germination were those that hydrated to a higher moisture content during the accelerated ageing process.

Generally, accelerated ageing and soaking in distilled water were highly correlated in case of all the parameters measured ($r = 0.9$). The number of normal seedlings after accelerated ageing were correlated with normal seedlings after 12 h soaking in distilled water ($r = 0.9$). Similarly abnormal seedlings after accelerated ageing were correlated with abnormal seedlings after soaking ($r = 0.7$).

However, hypocotyl length and number of ungerminated seeds after accelerated ageing were not correlated to hypocotyl length and number of ungerminated seeds after soaking ($r = 0.4$). The performance of the small yellow seeded Indian cultivar Pb-1 was better than the native small black seeded cultivar Kalitur. Kalitur had poor initial germination and was very susceptible to accelerated ageing.

The American cultivars (Essex and Stonewell) produced a similar number of normal seedlings, but medium seeded cultivar Stonewell had a longer hypocotyl length ($P < 0.03$). The cultivar Opale was superior to Gemma, both cultivars were large seeded.

6.3.1.1. Normal seedlings

American cultivars:- Figure 6.1a show that the American cultivars Essex (smaller seeded) and Stonewell (medium seeded) initially produced similar number of normal seedlings. Accelerated ageing or soaking in distilled water reduced the number of normal seedlings by 15% or 18% for Essex, and by 12% or 16% respectively in Stonewell compared to the unaged control ($P < 0.03$). There were no great differences in the number of normal seedlings between the cultivars after accelerated ageing or after soaking in distilled water (Figure 6.1a).

French cultivars:- Compared to the control, accelerated ageing or soaking in distilled water reduced the number of normal seedlings in larger seeded Opale by 24% or 33% respectively and in Gemma by 52% or 71% respectively ($P < 0.02$). When seeds were germinated after 12 h imbibition then the number of normal seedlings obtained from Opale and Gemma was 12% or 39% lower compared to those produced from seeds subjected to 4 days accelerated ageing ($P = 0.02$).

Indian cultivars:- Control seed samples of the smaller seeded Kalitur (black seed coat) produced 14% fewer normal seedlings compared to the yellow smaller seeded Pb-1 ($P = 0.04$). Accelerated ageing or soaking reduced the number of normal seedlings by 29% or 54% respectively in case of the cultivar Kalitur compared to the control ($P < 0.01$).

The cultivar Pb-1 showed a strong resistance to accelerated ageing or soaking in distilled water and hence the number of normal seedlings decreased by only 6% (Figure 6.1a). Compared to accelerated ageing, the number of normal seedlings from cultivar Kalitur was 50% lower after soaking of the seeds for 12 h in distilled water ($P < 0.01$).

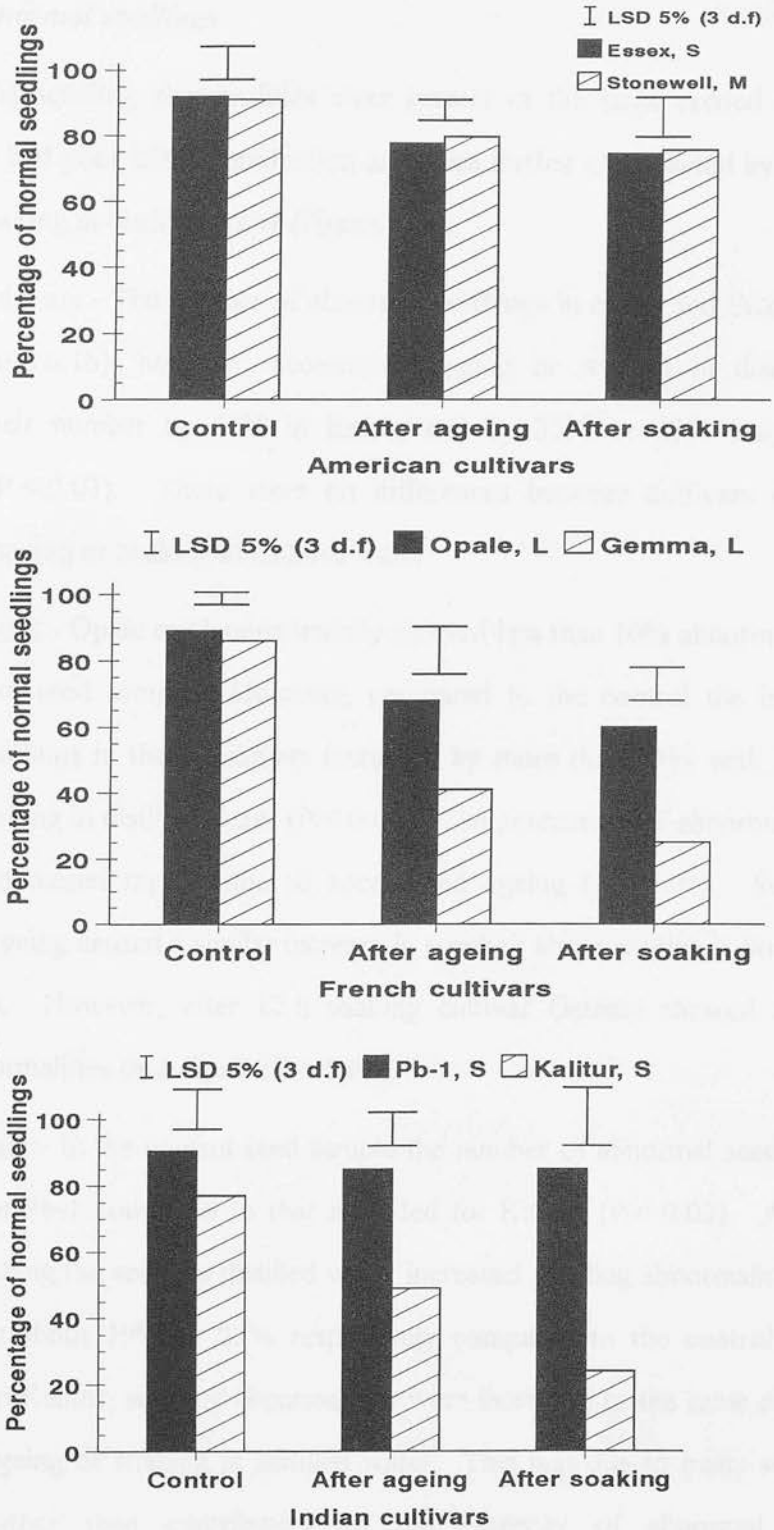


Figure 6.1a. Relative effect of accelerated ageing and soaking in distilled water on normal seedlings in (S) small (M) medium, and (L) large seeded soybean cultivars.

6.3.1.2. Abnormal seedlings

Generally the seedling abnormalities were greater in the large seeded cultivars or cultivar that had poor initial germination and were further exacerbated by accelerated ageing or soaking in distilled water (Figure 6.1b).

American cultivars:- The number of abnormal seedlings in Essex and Stonewell were similar (Figure 6.1b), however, accelerated ageing or soaking in distilled water increased their number by 40% in Essex, and by 35% or 43% respectively in Stonewell ($P < 0.03$). There were no differences between cultivars or between accelerated ageing or soaking in distilled water.

French cultivars:- Opale or Gemma initially showed less than 10% abnormal seedlings in the control seed sample. However, compared to the control the incidence of abnormal seedlings in these cultivars increased by more than 75% with accelerated ageing or soaking in distilled water ($P < 0.02$). The percentage of abnormal seedlings in Gemma increased rapidly due to accelerated ageing ($P < 0.04$). Soaking and accelerated ageing caused a similar increase in seedling abnormalities in both cultivars (Figure 6.1b). However, after 12 h soaking cultivar Gemma showed 20% more seedling abnormalities than Opale ($P = 0.03$).

Indian cultivars:- In the control seed sample the number of abnormal seedlings were 40% lower in Pb-1 compared to that recorded for Kalitur ($P = 0.02$). Accelerated ageing or soaking the seeds in distilled water increased seedling abnormalities in Pb-1 or Kalitur by about 19% or 23% respectively compared to the control treatment ($P < 0.05$). In Kalitur, seedling abnormalities were increased to the same degree with accelerated ageing or soaking in distilled water. This was due to many seeds being non viable rather than contributing to the category of abnormal seedlings (Figure 6.1b).

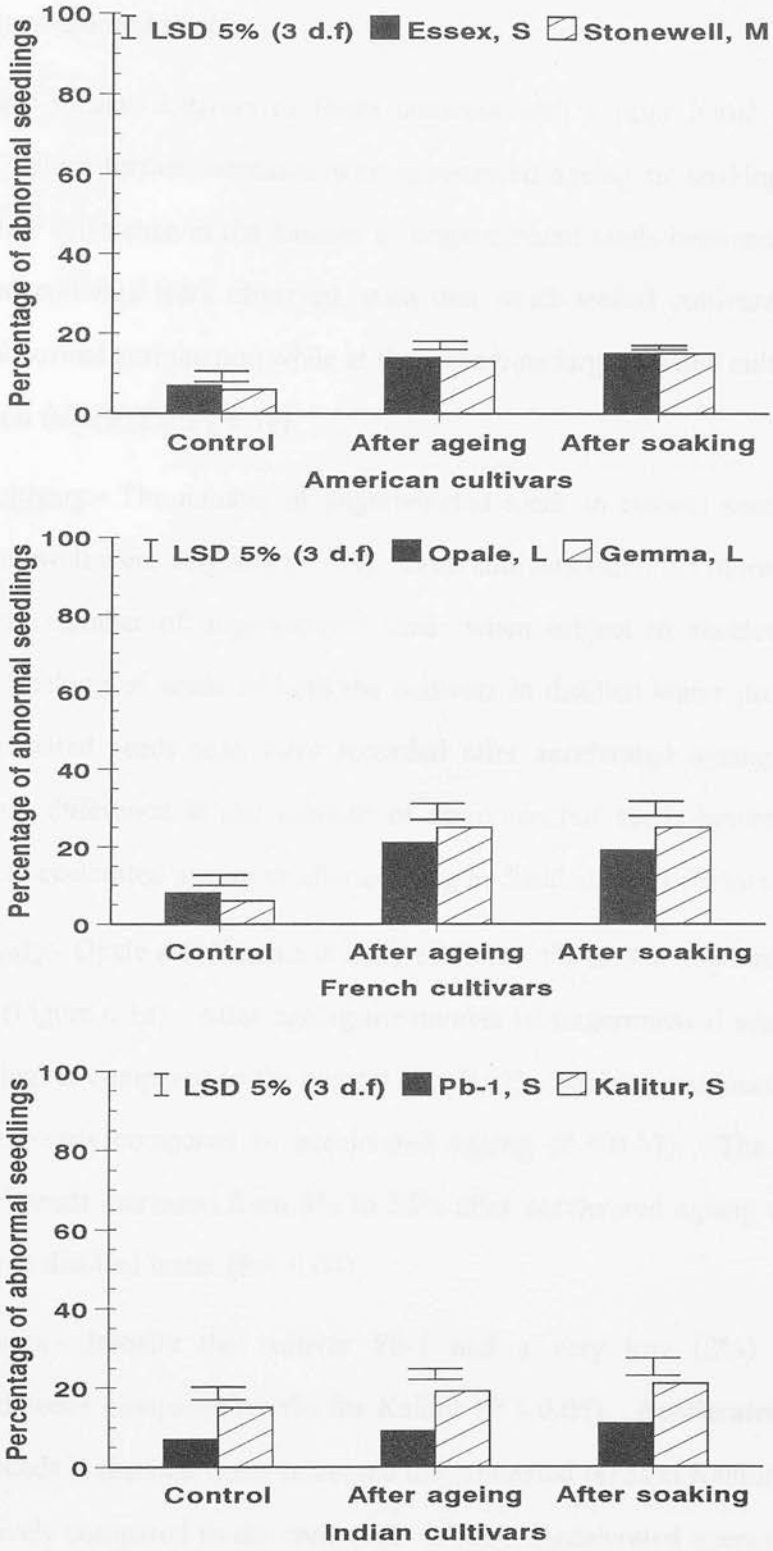


Figure 6.1b. Relative effect of accelerated ageing and soaking in distilled water on abnormal seedlings in (S) small (M) medium, and (L) large seeded soybean cultivars.

6.3.1.3. Ungerminated seeds

For the larger seeded cultivars or those cultivars with a poor initial germination, germination failure further increased with accelerated ageing or soaking in distilled water. A clear difference in the number of ungerminated seeds between smaller and larger seeded cultivars were observed, such that small seeded cultivars showed an increase in abnormal germination while at the same time larger seeded cultivars tended to germination failure (Figure 6.1c).

American cultivars:- The number of ungerminated seeds in control seed samples of Essex or Stonewell were very low (< 4%). Both cultivars exhibited more than a 50% increase in the number of ungerminated seeds when subject to accelerated ageing ($P < 0.01$). Soaking of seeds of both the cultivars in distilled water produced 20% more ungerminated seeds than were recorded after accelerated ageing ($P = 0.03$). There was no difference in the number of ungerminated seeds between the two cultivars after accelerated ageing or after soaking in distilled water (Figure 6.1c).

French cultivars:- Opale and Gemma initially exhibited 4% or 8% ungerminated seeds respectively (Figure 6.1c). After ageing the number of ungerminated seeds in Opale was 4 times higher compared to the control ($P = 0.01$). Soaking produced 50% more ungerminated seeds compared to accelerated ageing ($P < 0.01$). The number of ungerminated seeds increased from 8% to 35% after accelerated ageing and to 55% after soaking in distilled water ($P < 0.04$).

Indian cultivars:- Initially the cultivar Pb-1 had a very low (2%) number of ungerminated seeds compared to 8% for Kalitur ($P < 0.05$). Accelerated ageing or soaking the seeds in distilled water increased ungerminated seeds in Kalitur by 55% or 85% respectively compared to the control ($P < 0.01$). Accelerated ageing or soaking in distilled water, however, produced little increase in the number of ungerminated seeds in Pb-1 (Figure 6.1c). Seed death in small seeded cultivar Kalitur was greater

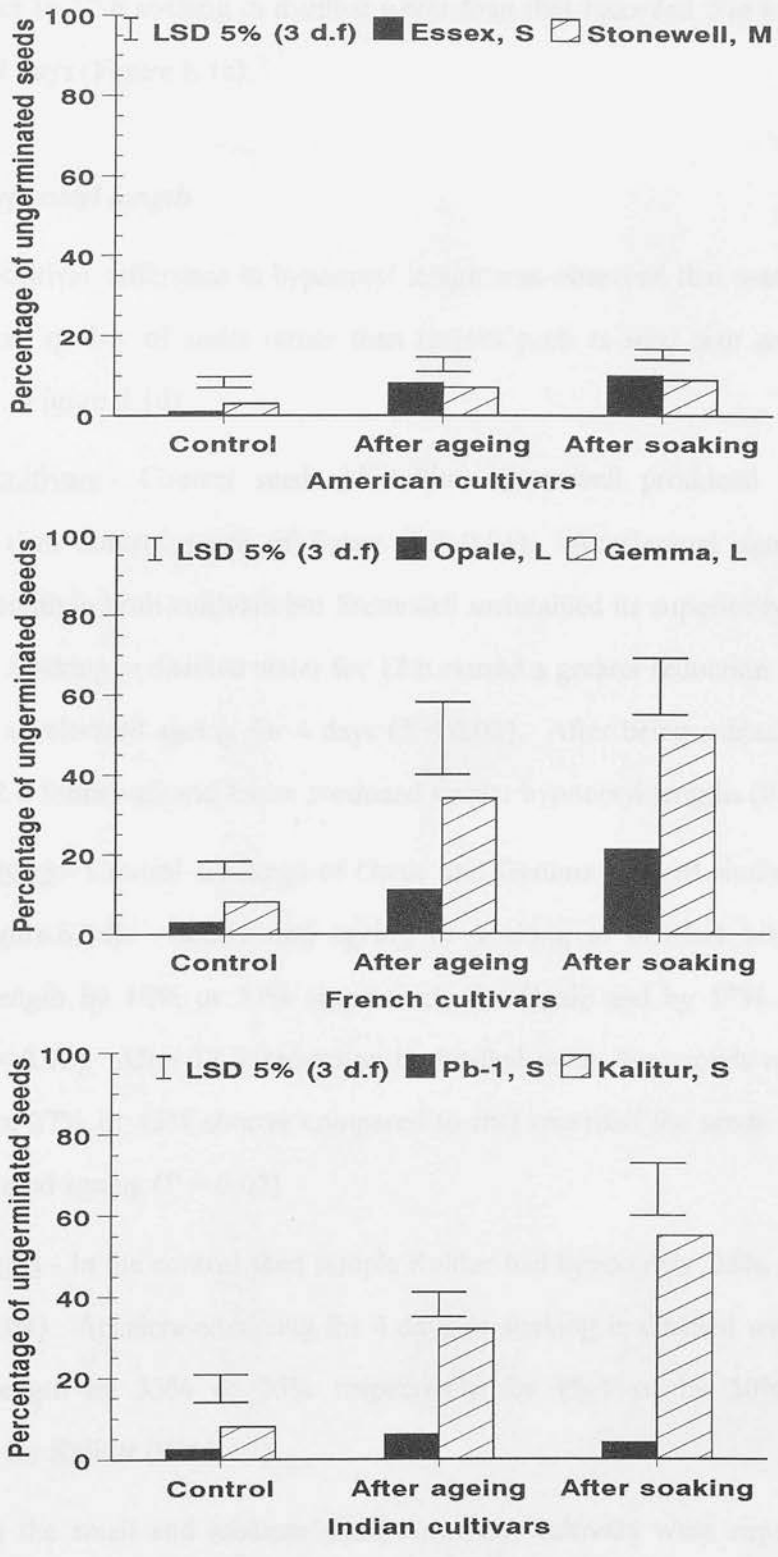


Figure 6.1c. Relative effect of accelerated ageing and soaking in distilled water on ungerminated seeds in (S) small (M) medium, and (L) large seeded soybean cultivars.

when subject to 12 h soaking in distilled water than that recorded due to accelerated ageing for 4 days (Figure 6.1c).

6.3.1.4. Hypocotyl length

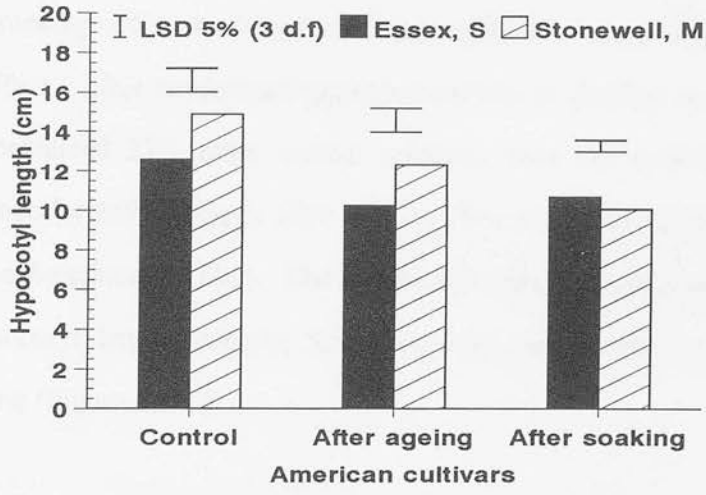
Significant cultivar difference in hypocotyl length was observed that was attributable to the overall quality of seeds rather than factors such as seed size and seed coat colour, etc. (Figure 6.1d).

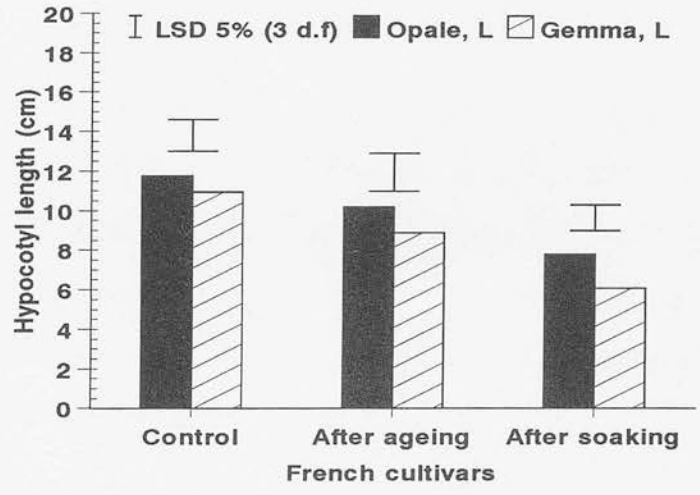
American cultivars:- Control seeds of cultivar Stonewell produced 16% longer hypocotyls than control seeds of Essex ($P < 0.01$). Accelerated ageing reduced hypocotyl length in both cultivars but Stonewell maintained its superiority over Essex ($P < 0.02$). Soaking in distilled water for 12 h caused a greater reduction in hypocotyl length than accelerated ageing for 4 days ($P < 0.02$). After being subject to distilled water for 12 h Stonewell and Essex produced similar hypocotyl lengths (Figure 6.1d).

French cultivars:- Control seedlings of Opale and Gemma showed similar hypocotyl lengths (Figure 6.1d). Accelerated ageing or soaking in distilled water reduced hypocotyl length by 16% or 33% respectively for Opale and by 17% or 41% for Gemma ($P < 0.02$). After 12 h immersion in distilled water hypocotyls of Opale and Gemma were 27% or 43% shorter compared to that recorded for seeds subject to 4 days accelerated ageing ($P = 0.02$).

Indian cultivars:- In the control seed sample Kalitur had hypocotyls 25% shorter than Pb-1 ($P = 0.01$). Accelerated ageing for 4 days or soaking in distilled water reduced hypocotyl length by 33% or 25% respectively for Pb-1 or by 50% and 43% respectively for Kalitur ($P < 0.01$).

Generally the small and medium sized American cultivars were superior to the large seeded French cultivars in germination and hypocotyl length. The controls of American cultivars Essex and Stonewell and the Indian cultivar Pb-1 had a similar





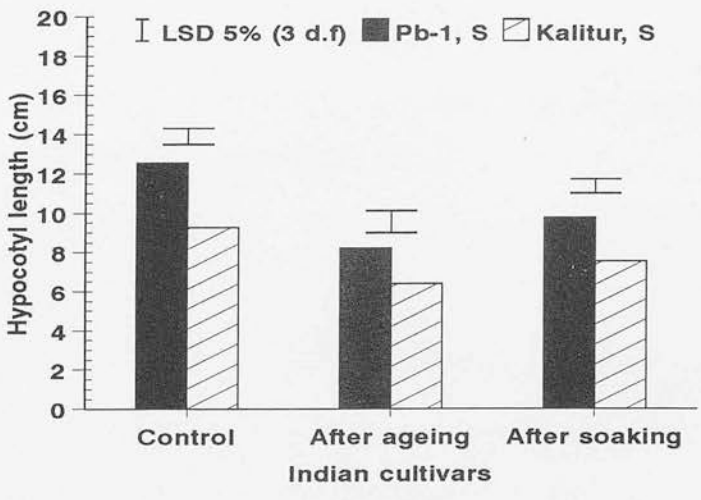


Figure 6.1d. Relative effect of accelerated ageing and soaking in distilled water on hypocotyl length in (S) small (M) medium, and (L) large seeded soybean cultivars.

germination percentage, but American cultivars exhibited longer hypocotyls than smaller seeded Pb-1. After accelerated ageing or soaking in distilled water the Indian cultivar Pb-1, produced 23% more normal seedlings than the American cultivars. However, after accelerated ageing or after soaking Pb-1 produced inferior hypocotyls than either of the American cultivars. The French cultivars showed good germination and longer hypocotyl length initially, but were very susceptible to soaking and accelerated ageing (Figure 6.1a-d).

6.3.2. Experiment 6.2

Comparative resistance of nine soybean cultivars to accelerated ageing and soaking injury.

During accelerated ageing the cultivars hydrated irrespective of their initial seed moisture contents. The larger seeded cultivars; Cumberland hydrated from 8 to 29.8%, Epps from 8.6 to 27.5%, Pixie from 9.3 to 29.4%, and Swat-84 from 9.3 to 31.4%; medium seeded cultivars; Woodworth hydrated from 9 to 29.9%, 79-W-220 hydrated from 9.4 to 30.5% and 80-B-4007 hydrated from 9 to 31.8%. However, the small seeded cultivar Weber hydrated from 8.7 to 26.7%.

The number of normal seedlings and shoot length was positively correlated ($r = 0.7$). Moreover, shoot fresh weight was positively correlated to shoot dry weight ($r = 0.9$). To some extent both shoot fresh and dry weights were related to shoot length ($r = 0.6$). In most cases cultivars that absorbed more moisture during accelerated ageing produced fewer normal seedlings when subsequently germinated, but this was not absolute (Figure 6.2a).

6.3.2.1. *Normal seedlings*

Control seed samples of Cumberland, Woodworth, and Epps exhibited between 88% to 93% normal seedlings (Figure 6.2a). However, the number of normal seedlings in the other cultivars varied from as low as 75% (80-B-4007 and Swat-84) to 85% in Pixie ($P < 0.05$).

During accelerated ageing the number of normal seedlings decreased by 38%, 21%, 29% and 37% in Cumberland, Epps, Pixie and Swat-84 respectively; by 33%, 39% and 37% in Woodworth, 79-W-220 and 80-B-4007 respectively, and 19% in Weber ($P < 0.01$). Weber (small seeds) and Woodworth (medium seeds) were the least affected compared to others ($P < 0.02$). However, Swat-84 and 80-B-4007

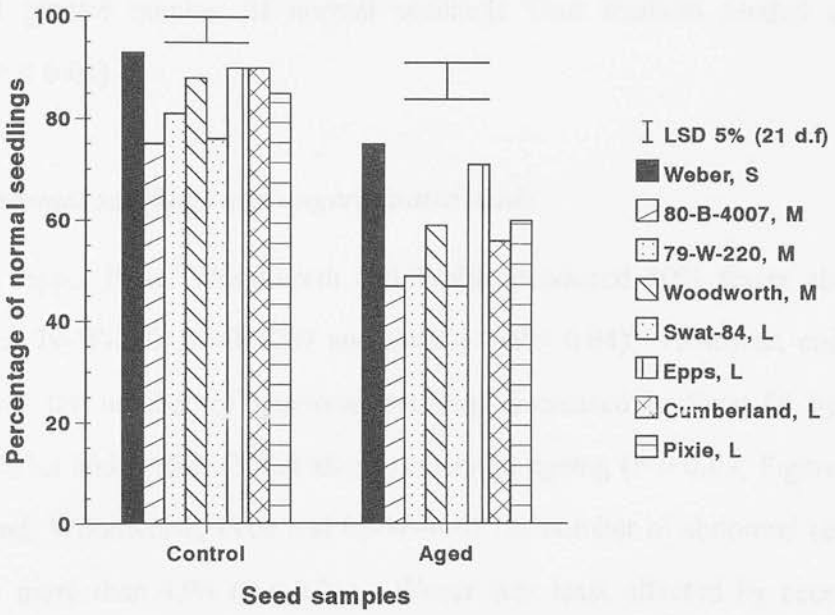


Figure 6.2a. Comparative effect of accelerated ageing on normal seedlings in eight soybean varieties obtained from Agricultural Research Station, Mingora, Pakistan (S, small seeded. M, medium seeded. L, large seeded).

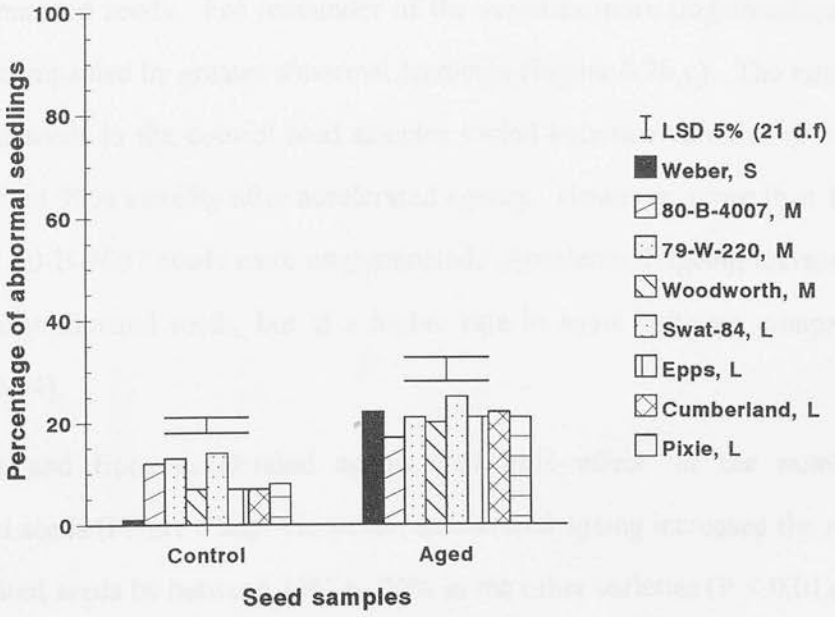


Figure 6.2b. Comparative effect of accelerated ageing on abnormal seedlings in eight soybean varieties obtained from Agricultural Research Station, Mingora, Pakistan (S, small seeded. M, medium seeded. L, large seeded).

(large seeded) decreased the most normal seedlings (Figure 6.2a). But Epps (large seeded) had greater number of normal seedlings than medium seeded cultivar 79-W-220 ($P < 0.01$).

6.3.2.2. *Abnormal seedlings and ungerminated seeds*

Cumberland, Epps, Pixie, Woodworth and Weber produced 50% fewer abnormal seedlings than 79-W-220, 80-B-4007 and Swat-84 ($P < 0.04$). However, compared to the control the number of abnormal seedlings increased in Swat-84 by 25%, 79-W-220 (29%) and Weber (34%) after accelerated ageing ($P = 0.03$; Figure 6.2b). In Cumberland, Woodworth, Pixie and 80-W-4007 the number of abnormal seedlings increased by more than 43% ($P < 0.01$). Weber was least affected by accelerated ageing ($P < 0.03$), and produced fewest ungerminated seeds (Figure 6.2b). During accelerated ageing seeds of Swat-84 and 80-B-4007 deteriorated more quickly than other varieties. However, Weber and Epps produced more abnormal seedlings, but fewer ungerminated seeds. For remainder of the varieties more ungerminated seeds were also accompanied by greater abnormal seedlings (Figure 6.2b,c). The number of ungerminated seeds in the control seed samples varied between varieties ($P < 0.05$). Weber exhibited 99% viability after accelerated ageing. However, more than 10% of Swat-84 and 80-B-4007 seeds were ungerminated. Accelerated ageing increased the number of ungerminated seeds, but at a higher rate in some cultivars compared to others ($P < 0.04$).

In Weber and Epps accelerated ageing had little effect on the number of ungerminated seeds (Figure 6.2c). However, accelerated ageing increased the number of ungerminated seeds by between 15% to 20% in the other varieties ($P < 0.01$).

6.3.2.3. *Shoot length*

Controls of cultivars Woodworth, Epps (large seeded) and 80-B-4007

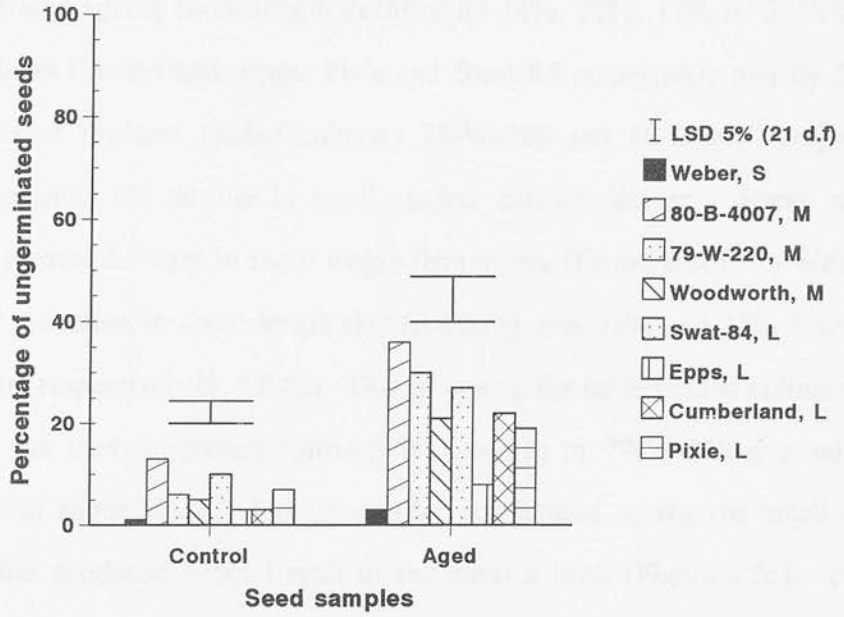


Figure 6.2c. Comparative effect of accelerated ageing on ungerminated seeds in eight soybean varieties obtained from Agricultural Research Station, Mingora, Pakistan (S, small seeded. M, medium seeded. L, large seeded).

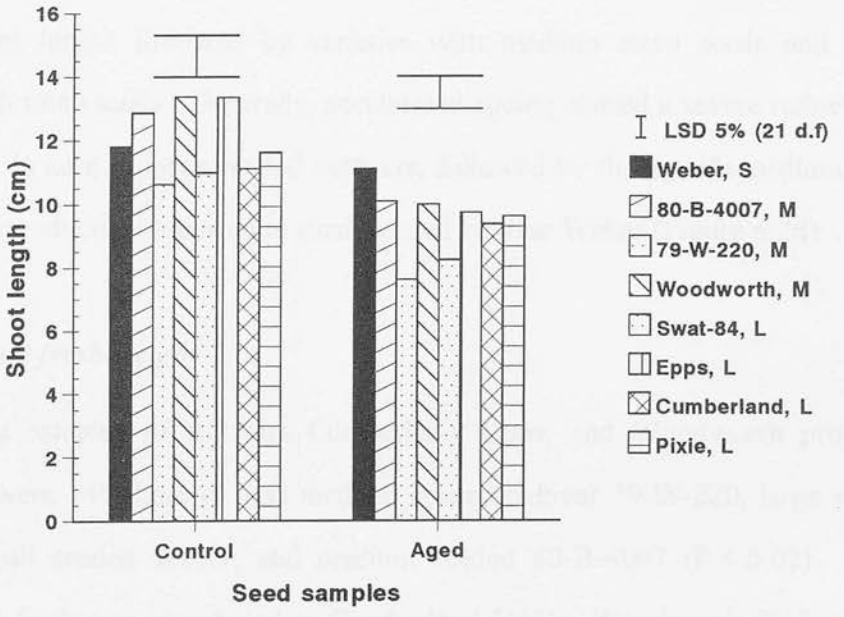


Figure 6.2d. Comparative effect of accelerated ageing on shoot length in eight soybean varieties obtained from Agricultural Research Station, Mingora, Pakistan (S, small seeded. M, medium seeded. L, large seeded).

(medium seeded) produced longer shoots compared to other cultivars ($P < 0.04$). After accelerated ageing shoot length declined by 14%, 27%, 17% or 25% in large seeded cultivars Cumberland, Epps, Pixie and Swat-84 respectively and by 28% or 21% in case of medium seeded cultivars 79-W-220 and 80-B-4007 respectively compared to only 6% decline in small seeded cultivar Weber. Some cultivars produced a greater decrease in shoot length than others (Figure 6.2d). In Weber and Cumberland reduction in shoot length due to ageing was 21% and 15% lower than other cultivars respectively ($P < 0.02$). Due to ageing the large seeded cultivars Epps or Swat-84 and medium seeded cultivars Woodworth or 79-W-220 showed about 25% decline in shoot length. However, after accelerated ageing the small seeded cultivar Weber produced shoot length to the control level (Figure 6.2d). For the control the average shoot length of small seeded Weber, medium seeded Woodworth and large seeded Epps was lower than medium seeded 80-B-4007, but after accelerated ageing shoot length of these cultivars exceeded the medium seeded cultivar 80-B-4007. Control seed samples of large seeded varieties produced the longest shoot length followed by varieties with medium sized seeds and finally varieties with small seeds. Generally, accelerated ageing caused a severe reduction in shoot length in case of large seeded cultivars, followed by those with medium seeds and minimum reduction occurred in small seeded cultivar Weber (Figure 6.2d).

6.3.2.4. *Shoot fresh weight*

Control seed samples of cultivars Cumberland, Epps, and Woodworth produced shoots that were 14% heavier than medium seeded cultivar 79-W-220, large seeded Swat-84, small seeded Weber, and medium seeded 80-B-4007 ($P < 0.02$). After ageing shoot fresh weight reduced in Cumberland (15%), Woodworth (25%), Epps (26%), Pixie (17%), 79-W-220 (28%), Swat-84 (25%), Weber (6%) and in 80-B-4007 by 21% ($P = 0.05$). After accelerated ageing shoot fresh weight of

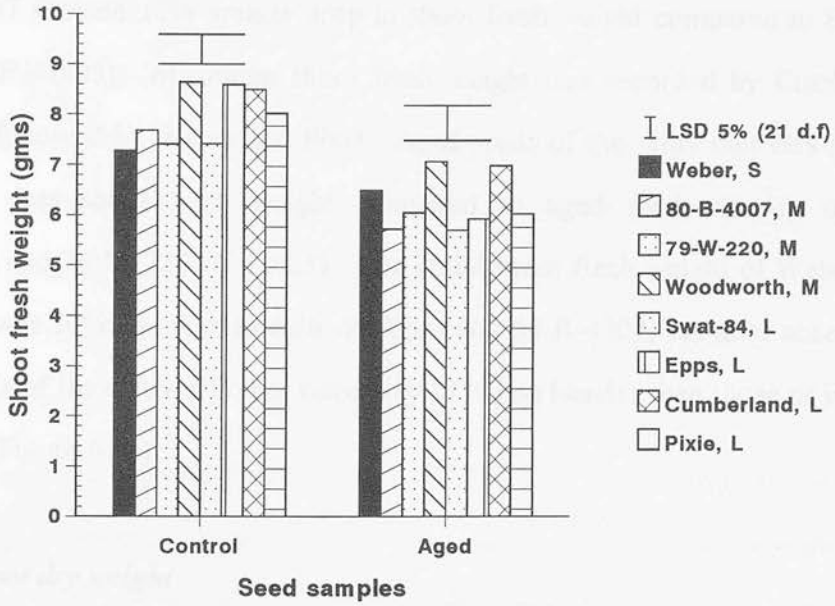


Figure 6.2e. Comparative effect of accelerated ageing on shoots fresh weight in eight soybean varieties obtained from Agricultural Research Station, Mingora, Pakistan (S, small seeded. M, medium seeded. L, large seeded).

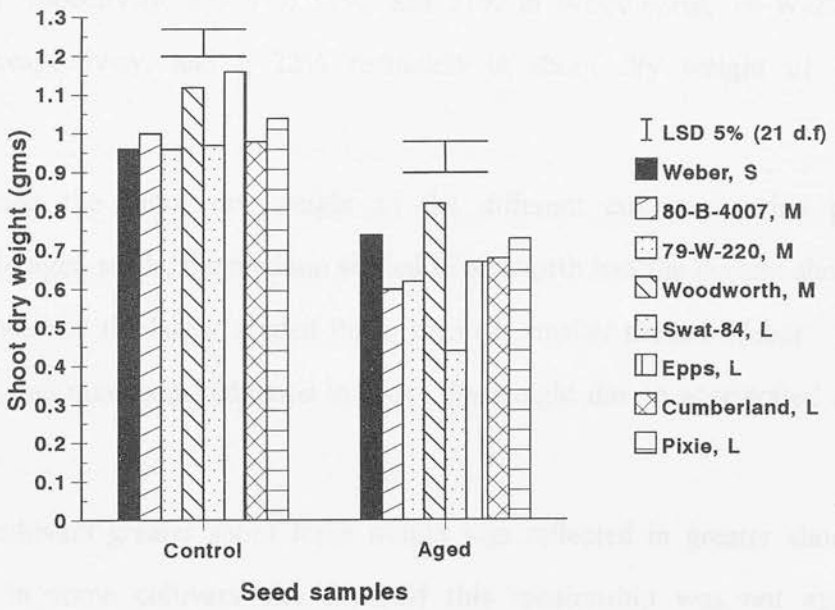


Figure 6.2f. Comparative effect of accelerated ageing on shoots dry weight in eight soybean varieties obtained from Agricultural Research Station, Mingora, Pakistan (S, small seeded. M, medium seeded. L, large seeded).

Cumberland and 79-W-220 was superior to others (Figure 6.2e). Some cultivars such as 80-B-4007 showed 10% greater drop in shoot fresh weight compared to Epps or 79-W-220 ($P < 0.05$). Maximum shoot fresh weight was recorded by Cumberland 79-W-220, followed by Weber and Pixie. Aged seeds of the other cultivars showed significantly less shoot fresh weight compared to aged seed samples of both Cumberland and 79-W-220 ($P < 0.03$). The initial shoot fresh weight of Weber with small seeds was 20% less than to cultivars Epps and 80-B-4007, but after accelerated ageing shoots of the cultivar Weber were only 11% less heavier than those of Epps or 80-B-4007 (Figure 6.2e).

6.3.2.5. *Shoot dry weight*

Initially the shoot dry weight of Woodworth and Epps were 19% greater than Pixie and about 25% heavier than the other cultivars ($P < 0.02$). Accelerated ageing reduced shoot dry weight by 26%, 42%, 30% and 54% in Cumberland, Epps, Pixie and Swat-84 respectively; by 27%, 35%, and 21% in Woodworth, 79-W-220 and 80-B-4007 respectively, and a 22% reduction in shoot dry weight of Weber ($P < 0.01$).

After ageing the shoot dry weight of the different cultivars varied greatly ($P < 0.03$). In aged seeds, the medium seeded Woodworth had the highest shoot dry weight, followed by the larger seeded Pixie, then the smaller seeded Weber. It was Swat-84 that had maximum reduction in shoot dry weight due to accelerated ageing (Figure 6.2f).

In most cultivars greater shoot fresh weight was reflected in greater shoot dry weight, but in some cultivars like Swat-84 this relationship was not apparent (Figure 6.2f).

6.3.3. Experiment 6.3

Comparative resistance of nine soybean cultivars to accelerated ageing and soaking injury.

The larger seeded Emerald, Morgan, and York hydrated from 7.6%, 13.25 and 11.45%, to 33.8%, 28.9% and 31.6% respectively; medium seeded Conrad, Linford and Kent hydrated from 7.1%, 10.7% and 11.4% to 29.4%, 27.9% and 31.4% respectively. Finally small seeded Manokin, Ripley and Stafford hydrated from 8.7%, 14% and 9.5% to 27.9%, 28.4% and 26.8% respectively.

Normal seedlings were positively correlated with shoot fresh weight ($r = 0.7$), but there was no correlation between germinability and solute leakage or between shoot fresh weight and solute leakage. However, individually, most cultivars with higher germinability or greater shoot fresh weight leaked less solutes (Figure 6.3a-f).

Generally cultivars with smaller seeds produced more normal seedlings and leaked less solutes than medium seeded cultivars. Medium seeded cultivars performed better than those with larger seeds, but this relationship was not absolute ($P < 0.03$). Larger seeded cultivars had superior shoot fresh weight than were produced by medium or smaller seeded cultivars, but this was also not absolute (Figure 6.3d). The larger seeded Emerald with a green seed coat had higher initial germinability, but showed reduced number of inferior normal seedlings after accelerated ageing. Cultivars with yellow seed coats were superior to those with whitish seed coat. In some cultivars germinability was attributable to differences in moisture absorption during accelerated ageing, however, this was not true in all cases.

6.3.3.1. Normal seedlings

Control seed samples of most of the cultivars produced a similar amount of normal seedlings. However, less normal seedlings were produced by larger seeded Morgan

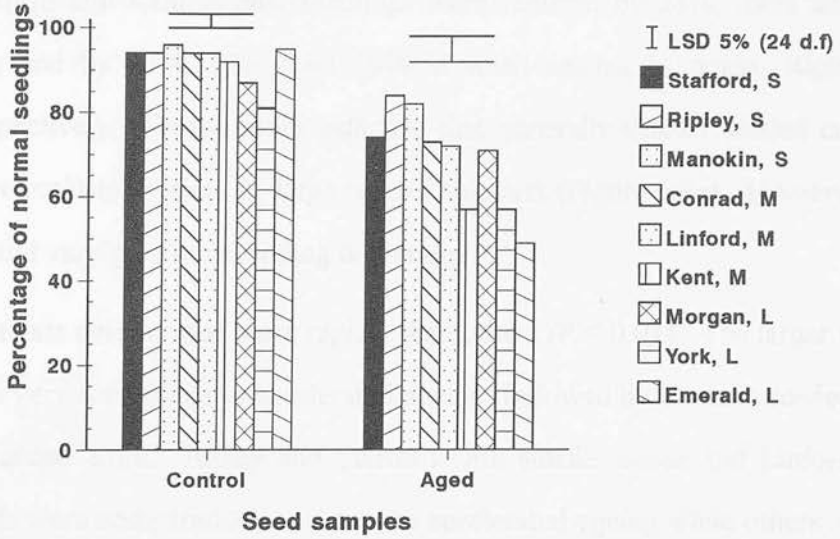


Figure 6.3a. Comparative effect of accelerated ageing on normal seedlings in nine soybean varieties obtained from Maryland, USA (S, small seeded. M, medium seeded. L, large seeded).

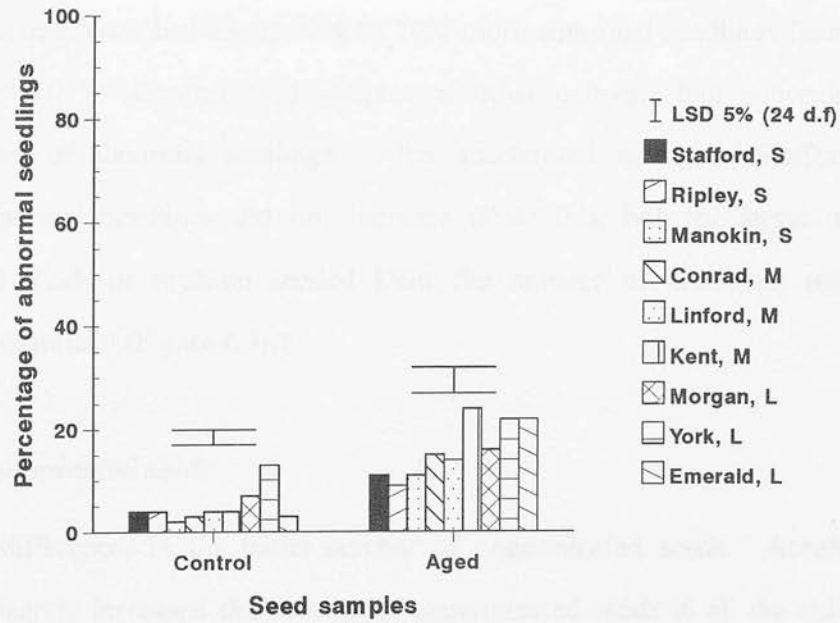


Figure 6.3b. Comparative effect of accelerated ageing on abnormal seedlings in nine soybean varieties obtained from Maryland, USA (S, small seeded. M, medium seeded. L, large seeded).

and York ($P < 0.03$). After ageing in Emerald, Morgan and York normal seedlings were reduced by 46%, 16% and 24% respectively. In medium seeded cultivars Conrad, Linford and Kent normal seedlings were reduced by 21%, 22% and 35% respectively, and by 23%, 10%, and 19% in small seeded Manokin, Ripley and Stafford respectively. These results indicated that generally smaller seeded cultivars performed better than medium or large seeded cultivars (Figure 6.3a). However, this observation did vary greatly depending on variety.

Some cultivars deteriorated more rapidly than others ($P < 0.01$) The larger seeded Emerald was very susceptible to accelerated ageing, followed by medium seeded Kent and larger seeded York. Ripley and Stafford with smaller seeds and Linford with medium seeds were comparatively resistant to accelerated ageing while others such as large seeded cultivar York was moderately susceptible (Figure 6.3a).

6.3.3.2. Seedling abnormalities

The larger seeded York had about 50% to 70% more abnormal seedlings than other cultivars ($P < 0.01$). Control seed samples of other cultivars had approximately similar number of abnormal seedlings. After accelerated ageing of Stafford and Manokin abnormal seedlings did not increase ($P < 0.04$), but for larger seeded Emerald and York or medium seeded Kent the number of abnormal seedlings increased substantially (Figure 6.3b).

6.3.3.3. Ungerminated seeds

There were differences in the initial number of ungerminated seeds. Accelerated ageing significantly increased the number of ungerminated seeds in all the cultivars. However, cultivars varied in the number of ungerminated seeds after ageing (Figure 6.3c).

Emerald was initially on top of the viability list, but produced more ungerminated seeds after being subject to accelerated ageing. Cultivars Kent and York also exhibited more ungerminated seeds after accelerated ageing. The smaller seeded Ripley and Manokin showed high viability and minimum ungerminated seeds initially, and less ungerminated seeds after accelerated ageing. The larger seeded cultivar Morgan and medium seeded cultivar Conrad produced similar number of ungerminated seeds both before and after ageing (Figure 6.3c).

6.3.3.4. Shoot fresh weight

There were great differences in smaller shoot fresh weight between the control seed samples of the cultivars ($P < 0.05$). Smaller seeded Ripley, Stafford and Manokin exhibited 10% or 16% greater shoot fresh weight compared to medium and larger seeded cultivars ($P < 0.02$).

Shoot fresh weight of the control of Morgan, York, Conrad and Linford were similar. The medium seeded Kent produced the lowest shoot fresh weight ($P < 0.01$). After ageing the shoot fresh weight in larger seeded cultivars Emerald, Morgan and York were reduced by 28%, 18% and 24% respectively (Figure 6.3d). However, shoot fresh weight in medium seeded Conrad, Linford and Kent were reduced by 26%, 11% and 15% and by 28%, 32%, and 20% in small seeded Manokin, Ripley and Stafford. This indicated that generally medium seeded cultivars Conrad, Linford and Kent experienced the least reduction in shoot fresh weight due to accelerated ageing. In addition, maintenance of a greater capacity for normal germination in small seeded cultivars was not a reflection of better performance in terms of seedling vigour as indicated by the large reduction of shoot fresh weight in these cultivars after ageing (Figure 6.3d).

The relationship between shoot fresh weight after ageing was unclear. The larger seeded Emerald, and smaller seeded Ripley, Manokin and Stafford produced the

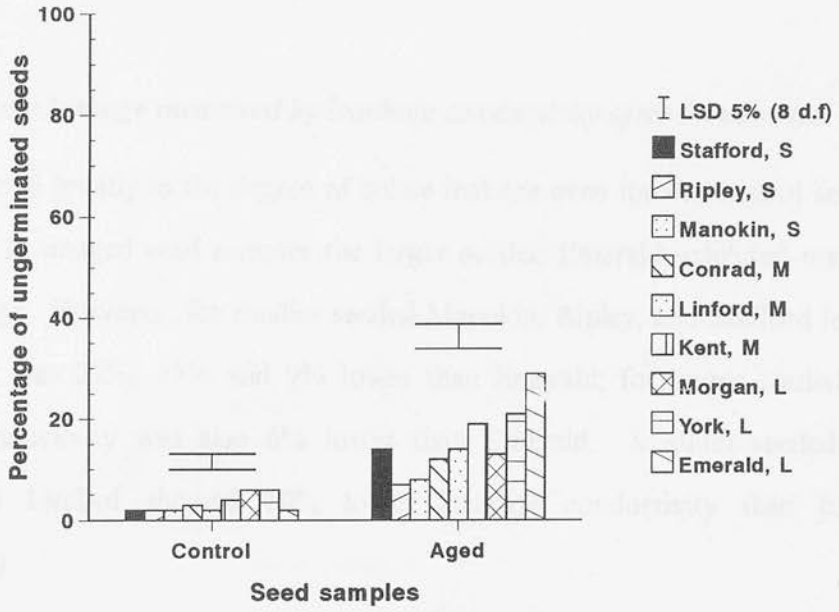


Figure 6.3c. Comparative effect of accelerated ageing on ungerminated seeds in nine soybean varieties obtained from Maryland, USA (S, small seeded. M, medium seeded. L, large seeded).

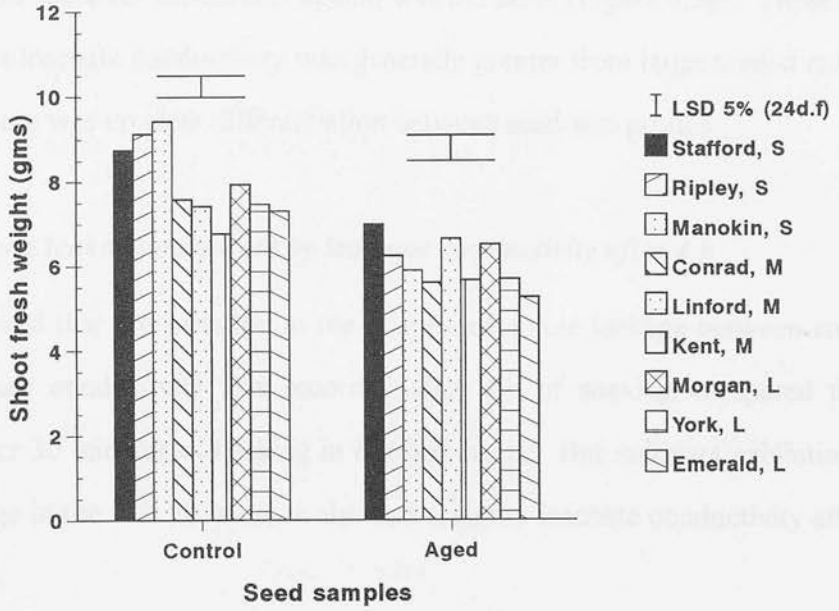


Figure 6.3d. Comparative effect of accelerated ageing on shoots fresh weight in nine soybean varieties obtained from Maryland, USA (S, small seeded. M, medium seeded. L, large seeded).

maximum decrease in shoot fresh weight due to ageing, followed by the medium seeded cultivar Conrad (Figure 6.3d).

6.3.3.5. Solute leakage measured by leachate conductivity after 30 minutes

Cultivars varied greatly in the degree of solute leakage even for the control seed lots ($P < 0.05$). In unaged seed samples the larger seeded Emerald exhibited maximum solute leakage. However, for smaller seeded Manokin, Ripley, and Stafford leachate conductivity was 22%, 17% and 9% lower than Emerald; for larger seeded York leachate conductivity was also 6% lower than Emerald. Medium seeded Kent, Conrad and Linford showed 10% lower leachate conductivity than Emerald (Figure 6.3e).

Compared to the control, ageing increased solute leakage in cultivars Emerald (16%), Morgan (10%), Kent (11%), York (8%), Stafford (7.5%) and Linford by 7.3% ($P < 0.02$), but by only 3% in Ripley and Conrad. However, in Manokin solute leakage before and after accelerated ageing was the same (Figure 6.3e). These results indicated that leachate conductivity was generally greater from large seeded cultivars, but in fact there was no clear differentiation between seed size groups.

6.3.3.6. Solute leakage measured by leachate conductivity after 4 h

It was observed that the variation in the amount of solute leakage between cultivars was less when conductivity was recorded after 4 h of soaking compared to that recorded after 30 minutes of soaking in distilled water. But cultivars exhibiting high solute leakage in the first 30 minutes also had a higher leachate conductivity after 4 h (Figure 6.3f).

Emerald, Kent and York produced the highest conductivity results. Compared to Emerald, Kent and York solute leakage was lower in case of the cultivars Ripley

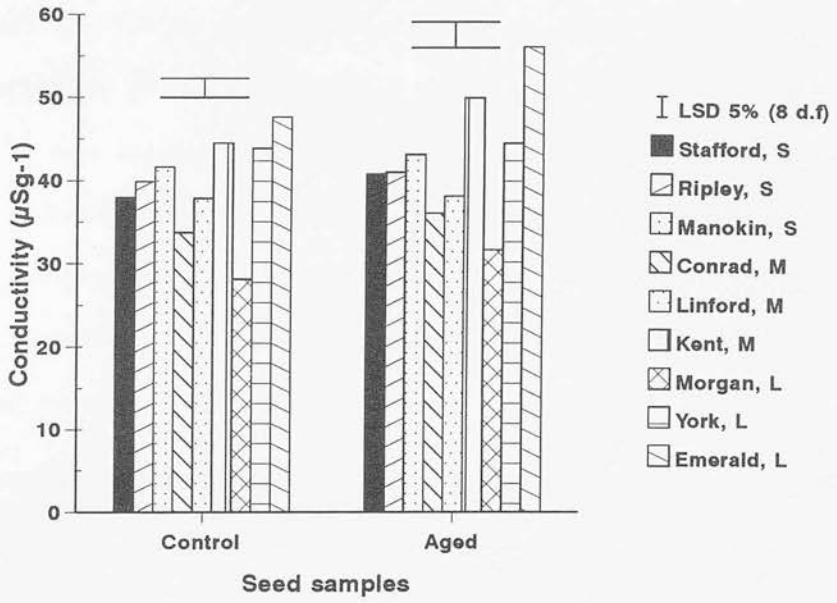


Figure 6.3e. Comparative effect of accelerated ageing on leachate conductivity recorded after 30 minutes soaking (S, small seeded. M, medium seeded. L, large seeded).

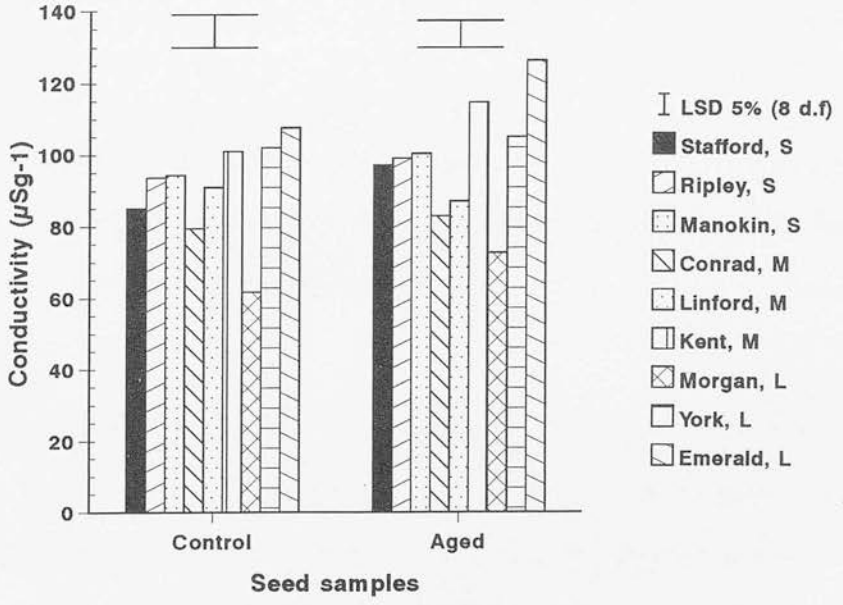


Figure 6.3f. Comparative effect of accelerated ageing on leachate conductivity recorded after four hours soaking (S, small seeded. M, medium seeded. L, large seeded).

(10%), Morgan (37%), Conrad (17%), Stafford (13%), Linford (21%) and Manokin ($P < 0.02$).

6.4. DISCUSSION

Soaking seed for 12 h in distilled water before a germination test provided an excellent separation of vigour within and across seed samples. Different soybean varieties followed a similar behavioural pattern of decline in germination if exposed to accelerated ageing or soaking in distilled water. Results indicated that cultivars with small seeds were less liable to soaking injury. This indicated that soybean varieties with small seeds would probably withstand more stressful field conditions (temperature, crust formation).

Cultivars with small seeds (Payne & Koszykowski, 1979; Dadson, 1982; Hill *et al.*, 1986a; Vanangamudi, 1988) and smooth seed coats (Singh & Seitia, 1974) have been reported to possess better storage potential. The results presented here indicated that hypocotyl growth is an important, yet often ignored aspect that must be considered in the evaluation of varieties for storage potential alongside other parameters. In soybeans the hypocotyl is probably the most critical organ affecting emergence under field conditions because it is responsible for lifting the cotyledons above the soil surface.

The French cultivars with larger seeds demonstrated a substantial loss in hypocotyl length due to accelerated ageing or soaking in distilled water. After ageing the larger seeds possibly produced hypocotyls that were incapable of lifting their large cotyledons above the soil surface.

Hypocotyl growth has been well studied in cotton by Wanjura & Buxton (1972). They found that under assumed field conditions in the greenhouse, hypocotyl elongation decreased and hypocotyl diameter increased with increasing soil hindrance (Hatfield and Egli, 1974). In soybean, soil temperature has been shown to have an effect on the rate of hypocotyl elongation, but the comparative effect of accelerated ageing or soaking has been often ignored.

Cultivars with small or medium seeds have been reported to exert a high emergence force compared to cultivars with larger seeds (Edward & Hartwig, 1971). The results (experiment 6.1) suggested that during germination soybean may alter its pattern of hypocotyl elongation especially due to barriers such as stones and hard clay soil aggregates. The rate of hypocotyl elongation may be dependent upon factors such as genotype, seed size, soil temperature, moisture availability and the resistance offered by soil during seedling emergence. Therefore, there may be no specific shape of hypocotyl or specific emergence "force" in case of soybean, as has been suggested earlier in case of cotton by Wanjura & Buxton (1972). In other words germination in soybean is epigeal and the shape of hypocotyl or force of emergence may be related to the size and shape of cotyledons, because the shape and size of cotyledons varies with genotype, seed size and soil environment.

This study has demonstrated that certain soybean varieties are better equipped against the hazards of unfavourable storage environment (experiment 6.2). After accelerated ageing cultivars with small seeds produced most normal seedlings, less seedling abnormalities, fewer ungerminated seeds and leaked fewer solutes into the imbibing media compared to cultivars with medium or large seeds (experiment 6.3).

Cultivars with medium sized seeds performed better than cultivars with larger seeds, but this was not absolute. Cultivars with yellow seed coats performed better to those with lighter colour seed coats. These results are consistent with the findings of Dassou & Kueneman (1984) who tested 35 genotypes for resistance to both field weathering and artificial weathering inside an incubator. It was reported that soybean genotypes with large seeds were susceptible to field weathering and deterioration in storage. However, they also observed that some genotypes with small seeds were susceptible to weathering in the field or ageing in the laboratory.

This means that confusion exists in the superiority of smaller over larger seeded cultivars as far as the rate of seed deterioration is concerned. Further investigation is

needed. However, before comparing genotypes with different seed characteristics it is important to make sure that all the genotypes have been exposed to similar growing conditions.

It is also important that seeds of the genotypes are theoretically at a similar quality level. Only then one can make realistic and conclusive comparisons for storage potential between genotypes. Some genotypes with small seeds may not be adapted to a specific set of growing conditions and therefore it does not make sense to compare such varieties with a better adapted variety.

Seed colour may also produce an important effect in such studies. A cultivar with smaller and pale coloured seeds should not be compared with a medium seeded yellow colour cultivar. Because the expression due to seed size is suppressed by the expression due to seed colour. Experiments 6.2 and 6.3 evaluated yellow seeded cultivars only. However, one cultivar with larger seeds, Emerald, had green seeds and was very susceptible to accelerated ageing.

Unlike the results presented by Dassou & Kueneman (1984) all cultivars with small seeds in this experiment proved their superiority over large and medium seeded cultivars. However, the superiority of cultivars with medium sized seeds over cultivars with larger seeds was not certain.

Results in experiment 6.3, showed that genotypes with small seeds leak fewer solutes. Hwang & Sung (1991) also observed less leakage in genotypes with smaller seeds and those genotypes coated with ethyl cellulose before soaking. The structure and thickness of the seed coat are regarded as the most important factors determining storability and the amount of soaking damage caused to the seeds (Potts *et al.*, 1978; Ragus, 1987). Hill *et al.* (1986a) reported that ruptured seed coats in soybean were caused by the cotyledons expanding against the seed coat.

Research findings of the above workers suggest that cultivars with small seeds may

have thicker seed coats compared to cultivars with larger seeds. Besides a genetic reason this behaviour may also be explained by source-sink relationship at the seed filling stage. For smaller seeds the source may be limited and therefore the cotyledons may not expand against capacity of the seed coat, hence the seed coats are compact. However, in case of larger seeds source may not be a limited factor causing the expansion of the cotyledons against the capacity of the seed coat and contribute towards thin seed coats.

It is suggested that both seed of high planting value and suitable expertise in planting are indispensable, if satisfactory crop stands are to be expected. Smaller soybean seeds or those with thicker or smooth seed coats are equipped to cope with mechanical damage during harvesting and postharvest handling. They also cope better with poor storage conditions. These seeds probably maintain the integrity of their seed membranes (Potts *et al.*, 1978; Koslanund & Delouche, 1987). For soybean seeds lacking these characteristics polyethylene glycol has been reported to be very useful in preventing imbibition damage especially in low vigour seed lots (Woodstock & Taylorson, 1981; Woodstock & Taoi, 1981; Seong *et al.*, 1988; Murray, 1989).

CHAPTER 7

GENERAL DISCUSSION

Low seed vigour in soybean often results in poor seedling emergence and crop stand especially when seeds are planted in adverse field conditions. Poor stand establishment is a major constraint to the expansion of soybean cultivation in the tropics and subtropics (Nangju *et al.*, 1980). Tropical and subtropical seed storage is often practised in conditions of high temperature and high relative humidity environment. In addition, high soil temperature has been cited as a factor contributing to poor crop establishment (Emerson, 1982).

As mentioned earlier, this thesis has been concerned with the major environmental and seed quality factors that affect seed vigour and viability in soybean. The relationship of laboratory germination and tetrazolium tests to field emergence in aged seeds has been established. In addition, the effect of ageing and of the rate of water uptake on germinability has been studied. An attempt has been made to control water uptake injury by slowing down the initial inrush of water into the seed during imbibition and to enhance germination, using polyethylene glycol (PEG). Finally, particular emphasis has been given to identify characters of cultivars that store well and are resistant to soaking injury.

7.1. LABORATORY TESTS AS ESTIMATORS OF FIELD EMERGENCE

The laboratory germination and tetrazolium chloride topographic staining tests were good estimates of field emergence, as long as 80% or more normal seedlings were produced in rolled paper towels under ideal laboratory conditions. However, seed

samples with less than 80% normal seedlings in the laboratory tests exhibited even lower emergence under field conditions (Agricultural Research Station, Mingora, Pakistan). At 35°C, germination percentage and seedling fresh weight in rolled paper towels decreased that can be attributed to high solute leakage compared to germination and solute leakage at 25°C. Maximum seedling fresh weight was obtained at 30°C (experiment 4.1).

A variety of physiological and biochemical laboratory evaluations have been used to estimate the performance of high vigour seed lots under field conditions. However, any variation in field conditions can affect seedling emergence (Kulik & Yaklich, 1982). An accurate estimate of field emergence was dependent on the physiological state of the seed and germination temperature. However, if a germination test was performed at 35°C, then the number of normal seedlings produced from a seed sample aged for 4 days was 25% lower compared to that actually obtained under field conditions. After 8 days of accelerated ageing none of the seeds germinated at 35°C. A laboratory germination test performed at 25°C produced a good estimate of field emergence.

Seed aged for 6, 8 or 10 days failed to emerge under field conditions. Accelerated ageing encourages loss of membrane integrity and results in high solute leakage during germination leading to a poor emergence "force" under field conditions. A rapid decline in seedling fresh weight of aged seeds in the laboratory also support this hypothesis. Researchers have reported a decrease in emergence "force" of seedlings that developed from low vigour seeds (Pinthus & Kimel, 1979). The seeds leaked more solutes at high soaking temperature in the laboratory. Therefore, in case of aged seeds, high soil temperature (between 35°C or 40°C) may be associated with high solute leakage and poor emergence "force".

As with the tetrazolium test (Sripichitt *et al.*, 1988) measurement of seed leachate conductivity has long been used in some species notably peas (Powell & Matthews,

1981) and more recently in long beans, *Vigna sesquipedalis* (Abdullah *et al.*, 1992). Yaklich *et al.* (1979) also studied the relationship of seed viability and solute leakage, and obtained a correlation between these two variables. These results also support the findings of Yaklich *et al.* (1979). However, seedbed conditions (Leudders & Burris, 1979; Rathore *et al.*, 1983), soil temperature (Bharati *et al.*, 1983), soil moisture (Peske, 1983), initial quality of the seeds (Gove, 1965) and initial seed moisture (Bartsch *et al.*, 1986) all affect subsequent germination.

The ultimate purpose of most laboratory tests of seed quality is to evaluate the suitability of seed lots for planting in the field. However, frequent inconsistency of results from seed quality tests to field emergence has frustrated efforts in the tropical and subtropical countries. For example, in various investigations soybean field emergence has been found to be best related to conductivity of seed exudate (Oliveira *et al.*, 1984), to the accelerated ageing or cold tests (Kulik & Yaklich, 1982), or to a combination of seed quality tests (Yaklich & Kulik, 1979).

Some of these inconsistencies may be due to the use of different procedures; however, most can probably be attributed to variations in the planting environment. This suggestion recognises that different seed lot characteristics may be better related to performance in specific field situations. Consequently it is proposed that seed quality tests should stimulate the stresses imposed by different soil environments. Woodstock (1973) made similar suggestions.

If laboratory tests are performed under a particular set of conditions they can be expected only to estimate emergence under a certain range of field conditions. To obtain a more realistic estimate of field emergence, laboratory germination, conductivity and tetrazolium topographic staining tests should be standardised separately for a variety of seed samples and field conditions. The standardisation procedure may require a consideration of soil temperatures, soil type and seedbed condition together with an account taken of cultivars with different seed

characteristics such as seed size and seed coat structure. Useful recommendations for specific field conditions can only be made after a number of experiments are performed under a variety of field and laboratory conditions involving different soybean cultivars and different seed samples of each cultivar. Such an approach may lead to a specific laboratory test for specific field conditions. This would be highly desirable, because uncertainty of field emergence is a major problem hampering soybean production in the tropics and subtropics.

These results (experiment 4.1) have also shown that in case of low vigour seed sample normal field emergence is lower than the number of normal seedlings obtained under unstressed conditions in the laboratory. Therefore, instead of always conducting laboratory tests under ideal conditions it is suggested that the laboratory tests should be performed under stress conditions equivalent to those in the field.

The results also showed that laboratory germination, tetrazolium staining and the conductivity test are capable of estimating potential field emergence of a variety of seed samples. These results would be of value to researchers and farmers in Pakistan, who would like to rank different seed samples of the same variety or different varieties relative to specific field emergence. Once a desirable crop stand is obtained soybeans overcome environmental stresses during successive vegetative and reproductive stages (Troedson *et al.*, 1983).

An estimation or prediction of field performance are different terms. Estimation of high potential field emergence (equal to or greater than 90% field emergence) indicates that 90% of seedlings should emerge and establish under a range of field emergence. Whereas a prediction of 90% field emergence indicates that from a seed sample 90% of seeds will establish under any field conditions. Keeping in mind these two definitions it is concluded that the tests evaluated in this study were precise enough to accurately estimate field emergence in the environmental conditions at the Agricultural Research Station, Mingora, Pakistan. Their precision at any other

location in the country would need to be subject to further testing. However, the current study revealed that laboratory tests were a good estimate of field emergence if the seed lots were capable of producing more than 80% normal seedlings in rolled paper towels.

Tekrony & Egli (1977) concluded that due to the complex nature of vigour testing and the many tests available, a combination of the available laboratory tests may provide a useful estimate of field emergence capacity. However, this study suggests that the use of a variety of laboratory tests for one set of field conditions may lead to confusing results, because in using different laboratory tests there is a possibility that one test may overestimate and the other test underestimate field performance. For example in experiment 4.1 the tetrazolium chloride topographic staining test predicted significantly higher normal seedlings in seed samples which actually produced less than 80% normal seedlings in the laboratory. This provides further support to earlier suggestions, that future research should investigate and then recommend specific laboratory tests for a range of field conditions rather than recommending several tests for certain specific field conditions.

7.2. SEED MOISTURE, TEMPERATURE AND TIME DURING STORAGE

Loss of viability in storage is affected by a number of factors, however, in practice two of these factors, namely seed moisture content and storage temperature are most important (Dorworth & Christensen, 1968).

In experiment 4.2 seed samples of soybean at 8% moisture content produced more than 80% normal seedlings when germinated after storage at 35°C or 40°C. Seed samples with a moisture content of 12% stored well at 35°C, but at 40°C germinability decreased with storage time. However, seeds with a moisture content of 16% showed a rapid viability loss both at 35°C or 40°C.

The shift in storage behaviour of the seeds was associated with alteration in seed moisture content and storage temperature. Stewart & Bewley (1980) also observed similar behaviour of soybean seeds in storage. This effect of seed moisture and temperature during storage can be attributed to a quantitative increase in physiological reactions.

In tropical and subtropical countries storage conditions often fluctuate within the range studied in experiment 4.2 (Dadson, 1982). Dorworth & Christensen (1968) reported that the germination of soybean seeds declines rapidly if stored at 80% relative humidity at 30°C. However, it has been observed by Minor & Paschal (1982) and Potts *et al.* (1978) that soybean cultivars having hard seeds exhibit a better storage potential than cultivars with soft seeds due to a reduced rate of water uptake in a high relative humidity environment.

Most researchers regard seed moisture content as more important than storage temperature as far as seed longevity is concerned. However, if seeds are stored in unsealed conditions, as is normal in tropical and subtropical countries gain or loss in seed moisture and increase or decrease in ambient temperature both can be critical. Under practical storage in tropical and subtropical countries it is possible to dry the seeds and store them at 8% moisture content under sealed conditions, but reduction in storage temperature is beyond the reach of the farmers as cold stores are not available to them and for the seed companies it is very costly to run cold stores for long periods. The worst effects of higher temperature can be avoided by storage of seeds in sealed conditions at low moisture content (Justice & Bass, 1978).

These results suggest that deteriorative reactions in soybean are more rapid if the moisture content of the seeds is higher, and this does constitute a threat to longevity and hence survival in storage. From these results one might assume that deteriorative reactions in soybean seeds may only proceed at a higher moisture level (12% or 16%). However, Sripichitt *et al.* (1989) have detected reduction in seedling vigour and

viability after 18 months storage of soybean seeds at 8% moisture content.

Extrapolating results obtained in this study, if seeds were stored until next planting in tropics and subtropics, the rate at which seed deterioration occurs under sealed conditions is such that, at a moisture contents of 8% or lower, negligible deterioration would have occurred.

Under ambient storage conditions in the plains of Pakistan, loss of viability can be rapid leading to a reputation of soybean as being a notoriously difficult crop to store. Use of seeds of low viability often requires high and excessive seeding rates to compensate for poor emergence, a practice that is very expensive and unreliable. Poor germination of badly stored seed has frustrated efforts to expand soybean production in Pakistan. Considering the results of experiment 4.2, it is suggested that loss of seed vigour due to poor storage may lead to decreased field emergence and poorer subsequent seedling growth even if the seedlings succeed to emerge.

Soybean is among those species in which there is evidence that the deteriorative changes start in seeds well before death (Roberts, 1984). This means that the effect of seed vigour on emergence in soybean is more critical compared to other crops. Poor crop stands are more likely to reduce the final crop yield and quality of harvested seeds (Doss *et al.*, 1974). To secure high quality seeds for the next planting season choosing better sites or selecting good looking fields and taking extra care of seeds harvested from such fields in drying, threshing or transportation together with storage of the seeds under sealed conditions at below 8% seed moisture content may be helpful.

Ellis (1988) used his improved viability equation to predict the storage life of soybean seeds under various combinations of temperature (between 10°C to 50°C) and seed moisture contents (between 4.5% to 11.5%). Using the improved viability equation it was predicted that the viability of a soybean seed lot at 6.5% moisture

content that possess an initial viability of 95% will decline to 85%, if stored for 9 months at 30°C. Ellis (1988) illustrated the relationship of seed moisture with a maximum of 30°C, however, in June, July and August storage temperature in the plains of Pakistan, may well exceed 35°C

Future research should focus on a comparison of cultivars of different seed characters under sealed storage, because the storage behaviour of cultivars varies. The current results provide data that can be used in the improved viability equation of Ellis (1988) and predict storage life of soybean under a wider range of storage conditions. However, to make reliable predictions further investigations are needed. These investigations need to consider a wider range of storage temperatures.

7.3. Effect of initial seed moisture content and temperature on germinability

The initial moisture content of the seeds affects germinability depending upon its interaction with germination temperature. In the current study if seeds were germinated at 6% initial moisture content fewer normal seedlings and greater seedling abnormalities were produced accompanied by high solute leakage especially when germination temperature was high (35°C). However, seeds at a moisture content of 12% or 16% produced a greater number of normal seedlings, had fewer seedling abnormalities and showed lower solute leakage. These findings agree to the findings of Koslanund & Delouche (1987).

Higher solute leakage from aged seeds and from seeds with a lower moisture content indicated that in aged seeds membrane integrity was probably lost due to ageing or due to rapid water uptake in case of seeds with low moisture content. These results (experiment 4.3) support previous reports that losses in membrane integrity disturb the balance between physiological and biochemical processes during germination (Powell, 1988). Sorrells & Pappelis (1976) reported similar results.

However, from the results of experiment 4.3 it was not clear to what extent substrate loss alone affected germinability.

The number of normal seedlings decreased as solute leakage increased. Solute leakage was high if initial seed moisture content was low (6%). Hobbs & Obendorf (1972) reported decreased seedling vigour in case of soybean seeds that had a low initial moisture content. According to their findings soaking damage caused to seeds with low moisture was associated with reduced stover yield (broken pieces of straw after threshing) and decrease in plant height. They reported that cracks in the seed coats were responsible for poor performance due to excessive rate of water uptake. However, in the current experiment (experiment 4.3) water uptake injury caused to seeds at low initial moisture content was very well reflected in an increase in solute leakage.

It may be concluded that increased solute leakage in seeds with low initial moisture content may be due to minute cracks in the seed coat causing increased sub-cellular damage and reduced germinability. Duke & Harvey (1983) reported reduced germinability due to increased sub-cellular damage caused to soybean seeds. Early sub-cellular deterioration of soybean seeds may be due to enhanced rate of respiration.

The results of experiment 4.3 will have practical significance to soybean cultivation in Pakistan. Farmers could be recommended to take into account the initial moisture content of their planting seed which if too low would cause the seeds leak more solutes and thus create problems in seedling emergence and establishment. As a result of experiment 4.2 it has already been recommended that seeds should be maintained at 8% moisture content for better storage. That recommendation can be extended because of experiment 4.3 to include the fact that seeds at low moisture content (8% or less) should be allowed to hydrate to 16% or at least 12% moisture content before planting.

It is clear from the literature that investigations on these aspects have been made repeatedly, but firm conclusions have not been made. Future research needs to concentrate on a comprehensive experimental approach on a wide range of initial seed moisture contents, considering different cultivars and different germination temperatures.

7.4. EFFECT OF ETCHING ON GERMINABILITY AND SOLUTE LEAKAGE

The primary aim during harvest, postharvest handling and subsequent storage is to ensure the availability of soybean seeds having the potential to emerge rapidly and uniformly to establish a uniform stand of healthy and rapidly growing seedlings capable of producing a profitable high yield of high quality seeds. In the tropical and subtropical countries, however, soybean seeds are subject to substantial seed deterioration due to post-maturity preharvest weathering in the field and mechanical damage due to inappropriate postharvest handling (Tedia, 1982). Consequently, both emergence "force" and final emergence percentage are very low when these low vigour seeds are further subject to stressful planting conditions.

Therefore, in experiment 4.4 three cultivars with different seed sizes were evaluated after being subject to accelerated ageing or etching. The differences in germination between cultivars after being subject to accelerated ageing were attributed to differences in colour and size of the seeds. Differences in seed coat were detected as a result of differences in solute leakage. Unlike larger seeds, smaller seeds probably possess thicker seed coats and perhaps minimise the rate of water uptake that consequently results in less membrane damage and minimum solute leakage. The large seeded cultivar Gemma was very susceptible to accelerated ageing and leaked more solutes compared to cultivars with small seeds (Pb-1 and Essex), but unaged seeds of Pb-1 were more susceptible to etching than Gemma. This behaviour of seeds based on size could be problematic in screening varieties for tropical and subtropical

regions which demand cultivars that are more resistant to deterioration and are also capable of good germination under stress.

The observation that etched seeds had a lower germination compared to non etched seeds has previously been reported by Burchett *et al.* (1985). The small seeded Pb-1 was the cultivar least affected by accelerated ageing. Apart from smaller seed size, smoothness and waxyness of the seed coat may also have contributed to the good storage potential in Pb-1 (Singh & Seitia, 1974). Cultivars with such seed coats absorb less moisture from a high relative humidity environment compared to those possessing rough seed coats and therefore experience less deterioration. This was shown by the low conductivity measurement recorded for Pb-1.

Previous researchers have studied factors responsible for comparative mechanical damage caused to different soybean cultivars during postharvest handling. For example Tedia (1982) reported that soybean cultivars with large seeds were more susceptible to cracks in the seed coat than small seeded cultivars. However, in experiment 4.4, if large and small seeded cultivars received uniform cracks in the seed coat, then the large seeded cultivar exhibited better germinability than equivalent cultivars with small seeds. The poor performance of Gemma with larger seeds was associated with higher solute leakage that can be attributed to greater permeability of the seed coat. Hill *et al.* (1986b) have previously reported that the seed coat regulates solute leakage in soybean.

The current results showed that the phenomenon of high solute leakage from larger soybean seeds probably account for a major part of germination failure. These results (experiment 4.4) also suggest that if imbibed under similar conditions solute leakage from small seeded cultivars is lower compared to large seeded cultivars. This may explain the higher vigour of seedlings from small seeded cultivars. However, if the cotyledons or cotyledonary cells of either smaller or larger seeds are equally damaged, then success in germination is governed by seed size rather than by solute leakage. In

other words, seeds that leak less solutes may not necessarily exhibit better performance during germination compared to seeds that leak more solutes. Deteriorated or etched seeds failed to retain as many solutes as the controls suggesting that seed membranes were structurally damaged because of ageing or etching.

7.5. Effect of water uptake injury on germinability

Imbibition is the early stage of seed hydration that marks the period when the dry seed is first in contact with water and ends when the seed is fully hydrated and capable of initiating the process of germination as long as other essential environmental conditions are favourable. However, the sensitivity of seeds to rapid imbibition is determined by three factors; (a) the initial moisture content of the seed (b) the temperature of the medium, and (c) the rate at which water is taken up (Pollock, 1969).

In experiment 5.1 unaged and aged seeds produced fewer normal seedlings and exhibited delayed emergence if soaked in distilled water. Seeds soaked in 10% PEG also produced fewer normal seedlings and showed delayed emergence compared to the control. However, after soaking in PEG the number of normal seedlings was higher and emergence occurred earlier compared to seeds soaked in distilled water. Aged seeds produced the same number of normal seedlings if soaked in 25% PEG for 5 h, but 10 h soaking decreased the number of normal seedlings.

Many studies have attempted to relate the physiological responses of the soybean seed to the rate of imbibition. Parrish & Leopold (1977) found that the initial stage of imbibition culminated in a wetting of the seed coat and release of adsorbed gases. Others have shown that different solutes rapidly leak from (soybean) seeds during imbibition and this leakage is more pronounced if the seed is deteriorated due to ageing (Schoettle & Leopold, 1984). Results from the present experiment have also

shown that germinability in aged seeds is reduced more than in unaged seeds when subjected to soaking injury. McDonald *et al.* (1988b) reported that the seed coat plays a vital role in regulating seed water uptake.

A decrease in the number of normal seedlings after soaking in distilled water may be due to soaking injury that occurs after sudden inrush of water into the seed. Because the seeds soaked for 5 h in 25% PEG, probably leaked fewer solutes and retained the initial higher number of normal seedlings, decrease in the number of normal seedlings after 10 h soaking in 25% PEG was possibly due to anoxia as solute leakage in 25% PEG was expected to be negligible.

Matthews & Collins (1974) reported that resistant barley seed samples showed little impairment in the respiratory processes after 24 h under anaerobic conditions. However, compared to barley, the seed coats of soybean are vulnerable and allow the embryonic part of the seed to absorb moisture rapidly and respire at a higher rate which under submerged conditions may cause anoxia followed by cellular death. Low rates of oxygen uptake during imbibition of aged seeds in corn (*Zea mays* L.) was reported by Woodstock & Grabe (1967), but in soybean both embryonic and non embryonic parts of the seeds exhibited depressed oxygen consumption (Parrish & Leopold, 1977). Moreover, it was reported that the consumption of embryonic oxygen decreased as a result of seed deterioration.

Aged soybean seeds or seeds that were subjected to anaerobic conditions exhibited delayed emergence. Delayed emergence indicated poor emergence "force". This type of situation occurs in the tropics as reported by Tedia (1982) where severe soaking injury hampers emergence of seedlings because the seedlings exhibit poor emergence "force". These results (experiment 5.1) also indicated that soybean seeds may be susceptible to saturated soil conditions before emergence. Seeds may either suffer from soaking injury under temporary saturated conditions or if resistant to soaking injury, the seeds could suffer from anoxia. However, the modern soybean cultivars

are more susceptible to ageing and soaking injury than their wild ancestors (Lassim & Delouche, 1981). This indicates that there is a great variation in varietal response as far as storability and soaking injury are concerned.

Woodstock & Taoi (1981) reported no water uptake injury after soaking high vigour soybean seeds for 1 h. However, in the current experiment if the seeds were submerged for longer periods (5 h or 10 h) then even high vigour seeds showed reduced germinability compared to the control. However, compared to soaking in distilled water, germinability was improved by slowing down the initial rate of water uptake with 25% polyethylene glycol (PEG). The PEG treatments prevented the abnormal growth caused by rapid water uptake. Accelerated ageing, increase in solute leakage and increase in time under submerged conditions all decreased germinability and delayed emergence. The reduced germinability of aged seeds after soaking in water compared to PEG indicated that some damage in membranes occurred with ageing, but imbibition in PEG was unable to overcome this damage entirely. The results (experiment 5.1) also showed that 5 h soaking in 25% PEG maintained the number of normal seedlings produced by aged seeds at the same level as the control. This suggest that some electrolyte leakage may occur without any major effects on germinability. Results indicated that the cell membranes of the lower vigour seeds cannot tolerate rapid hydration in the early stages of imbibition.

After slow imbibition in 25% PEG, it is possible, to improve germinability that would have normally been poor if aged seeds were imbibed in water. The beneficial effect of slowly imbibing the seeds in PEG rather than in water suggested that a similar beneficial effect may be obtained by equilibrating the seeds to higher moisture content before germination in the field. These results will be useful for soybean growing in Pakistan.

Farmers are cautioned to avoid the risk of exposing soybean to saturated soil moisture conditions before emergence of the seedlings. Both short term and long

term saturated soil conditions can be damaging to successful germination and emergence. Seeds may either suffer from soaking injury in the initial hours of imbibition or lack of oxygen (anoxia) if saturated soil conditions lasts for longer. Even if seeds survive, emergent seedlings may be weak. A detailed investigation based on similar approaches needs to be made under a range of field conditions in Pakistan.

7.6. ELEVATING GERMINABILITY USING POLYETHYLENE GLYCOL (PEG)

Pre-planting equilibration to a higher moisture level was useful in avoiding soaking injury and enhancing germination (experiment 5.2). Slow imbibition in 25% PEG improved germinability of low vigour seeds by between 10% and 20%. Unaged and aged seeds imbibed in distilled water reached 50% germination more quickly, but for aged seeds this was accompanied by a decrease in normal seedlings when imbibition occurred in distilled water. Differences in the time to 50% germination could be attributed to the efficiency of pre-imbibed seeds. It is believed that repair mechanisms may be involved during slow imbibition of aged seeds in 25% PEG (Tilden & West, 1985).

Cellular membranes play a major role in seed deterioration. For example, Powell & Matthews (1978) reported that membrane damage during early imbibition in peas was critical due to its effects on solute leakage and germinability. It was suggested that the injury caused to seed membranes can be minimised by slowing the initial rate of water uptake.

The control of initial rate of water uptake using 25% PEG was also evaluated for its effects on shoot length, shoot fresh weight and shoot dry weight. Shoot length, shoot fresh weight and shoot dry weights of aged seeds were improved by slowing the rate of initial uptake of water. However, a slower rate of water uptake did not have any effect on shoot length, shoot fresh weight and shoot dry weight in high vigour

seed samples (experiment 5.3). This indicates that some kind of repair mechanism was probably involved in case of low vigour seeds attributable to slower water uptake.

The results indicate a close relationship between seed vigour and subsequent seedling development including shoot length, shoot fresh weight and shoot dry weight. A possible explanation of this association could be that the growth rate of seedlings is dependent on physiological and biochemical processes similar to those reported by Ching (1973). Ching (1973) reported that germination and seedling growth is affected by ageing. The findings of Seong *et al.* (1988) are also consistent with these findings.

In experiment 5.3 the differences between cultivars could be attributed to genetic differences and differences in seed size, because both the cultivars had similar initial germinability. In experiment 5.2, the effect of ageing was reversed when seeds were allowed to imbibe in 25% PEG for 24 h followed by germination. This was indirectly reflected in an increase in shoot length, shoot fresh weight and shoot dry weight in experiment 5.3.

Woodstock & Taylorson (1981) reported improved germination and growth when seeds were submerged in 30% PEG. The efficiency of 25% PEG in controlling or reversing the effects of soaking injury may be due to low vigour seeds being able to avoid death of cotyledonary cells under controlled imbibition.

Oliveira *et al.* (1984) reported higher cell death in soybean cotyledons that had absorbed moisture more quickly. Powell & Matthews (1978) reported similar results in peas (*Pisum sativum*) and Powell *et al.* (1986) reported similar results in French beans (*Phaseolus \vulgaris*). In French beans, cell death was responsible for high solute leakage and reduced germination and growth. This suggested that imbibition injury may be observed both in high and low vigour seeds and that this injury can be

avoided by slowly imbibing the seeds in PEG.

The results of experiment 5.2 and experiment 5.3, support the results of other workers by assuming cellular membranes as having a substantial role in seed deterioration (Parrish & Leopold, 1978; Simon, 1978).

Pre-planting equilibration to higher moisture content can be very helpful for improved crop stands in the plains of Pakistan. It may help to minimise the risk of poor crop stands which occur due to soil moisture loss from the root zone and may enable the seedling to use the available moisture sooner and extend roots deeper in the soil hence enable the seedlings to establish more rapidly. In saturated soil conditions pre-imbibed seeds will suffer less from soaking damage and emerge from the soil before being exposed to crust formation. However, the practicability of findings from this laboratory experiment requires confirmation under field conditions in Pakistan.

7.7. OSMOTIC STRESS AND SOYBEAN GERMINABILITY

Osmotic stress decreased the percentage of germinated seeds and seedling fresh weight of unaged seeds, but water stress applied at 5% and 10% PEG, increased the number of germinated seeds in aged seeds. Both unaged and aged seeds produced a greater seedling fresh weight if germinated in paper towels supplied with distilled water. The rate of water uptake and seedling fresh weight was reduced as the concentration of PEG, increased (experiment 5.4).

Increase in the germination percentage of aged seeds in paper towels supplied with PEG is probably due to reduced rate of water uptake preventing imbibition injury in some of the very delicate seeds. However, reduction in the fresh weight of the seedlings after PEG treatment was probably due to osmotic stress at a stage where the roots of the seedlings required moisture absorption at a higher rate to fulfil the demands of rapidly growing seedling. This has been previously reported that the early

growth of seedlings depend heavily on amounts of water taken up by the seedling (Hegarty, 1978).

The total amount and rate of water uptake during imbibition depend on the concentration of water around the seed. Seeds absorbed less moisture due to osmotic stress applied through PEG as has been previously shown by McDonald *et al.* (1988a). Ferriss *et al.* (1987) also reported reduced seedling growth under moisture stress conditions. This revealed that soybean seeds can initiate germination under a certain degree of moisture stress, but early seedling growth is negatively affected if the stress prevails. Under increased stress, seeds may imbibe water, but fail to germinate.

There was a close relationship between the speed of germination and subsequent development of seedlings. When water stress was extended to seedling development then seedling growth was drastically decreased. This indicated that slow imbibition may help in avoiding water uptake injury and enable the seed to successfully go through all the physiological and biochemical events that occur during germination, but maintenance of moisture stress thereafter may lead to less vigorous seedlings. Pinthus & Kimel (1979) drew similar conclusions after investigating speed of germination in soybean.

7.8. VARIETAL EVALUATION

In general, if atmospheric conditions in a seed store remain below 30°C and 70% relative humidity, successful storage of soybean seeds may be possible without out further control of the storage atmosphere (Delouche *et al.*, 1973). In the tropical and subtropical countries, however, these conditions are often exceeded leading to rapid seed deterioration. For example the germination of soybean fell rapidly when stored at 80% relative humidity at 30°C (Dorworth & Christensen, 1968). However, prolonged storage potential of soybean seeds has been reported in samples with hard

seeds and was attributed to the reduced rate of moisture absorption by these cultivars from the humid atmosphere (Potts *et al.*, 1978).

7.8.1. ACCELERATED AGEING VERSUS SOAKING IN DISTILLED WATER

In experiment 6.1 variation in the storage potential of different soybean cultivars was evaluated after accelerated ageing or after soaking in distilled water for 12 h followed by a germination test. Cultivars that hydrated at a higher rate during the accelerated ageing process were those that had larger seeds and consequently showed reduced germination after accelerated ageing or after soaking in distilled water. The phenomenon that soybean genotypes having small seeds are normally associated with thicker or hard seed coats and consequently exhibit the impermeability response at a higher rate compared to genotypes with larger seeds has been previously established by Choudry & Singh (1986).

This suggested that like accelerated ageing, germination after soaking or moisture uptake during accelerated ageing could also be used for screening soybean cultivars for storage potential. The cultivar with small seeds (Pb-1) showed comparative resistance to accelerated ageing and soaking in distilled water. However, the cultivar Essex with small seeds, cultivar Stonewell (medium seeded) and cultivar Opale (with large seeds) performed moderately well. One of the cultivars with small seeds (Kalitur) had less than 80% normal seedlings initially and was therefore very susceptible to accelerated ageing and soaking in distilled water. Hypocotyl length of cultivars with small or larger seeds decreased after accelerated ageing or after submergence in distilled water, but decrease in the hypocotyl length of cultivar Essex (small seeds) and Pb-1 (small seeds) occurred at a lower rate.

Results of experiment 6.1 are consistent with the results of Potts *et al.* (1978) who reported that the impermeable character of smaller soybean seeds contributed to maintenance of high seed viability and vigour under conditions that reduced the

viability and vigour of seeds with permeable seed coat or cultivars with large seeds. The cultivar Pb-1 is small seeded and Stonewell is medium seeded, but both these cultivars possess a waxy type seed coat.

The results of experiment 6.1 agree with the findings of Singh (1976) who stored soybean cultivars under ambient conditions in India. Singh (1976) reported that cultivars with larger seeds lost viability more quickly than cultivars with small seeds. This character was associated with smooth and hard waxy type seed coats in case of small seeded cultivars.

In experiment 6.1 both larger seeded and smaller seeded cultivars had higher initial germinability. However, after accelerated ageing it was revealed that high initial seed quality does not ensure good storability. Similar conclusions were reached by Singh *et al.* (1986).

Alongside the effect of accelerated ageing, the current experiment (experiment 6.1) investigated the effect that submergence in distilled water produced on germinability in soybean cultivars. Results showed that though accelerated ageing and soaking expose seeds to two different environments their effect on subsequent germinability is reasonably similar. Considering these results it is suggested that during selection of suitable cultivars for tropical and subtropical soybean growing areas, characteristics of the seed coat should be given priority compared to seed size. In other words a soybean cultivar with larger seeds, but a waxy type yellowish seed coat may show better storability and less soaking injury compared to a cultivar with small seeds and light coloured seed coats.

Besides those reported by Camacho & Munera (1986) tropical and subtropical countries need simple procedures of screening soybean cultivars for storability. Experiment 5.4 suggested that osmotic stress applied by means of PEG can be useful in screening cultivars that have the ability to perform better under moisture stress

conditions in Pakistan. However, results of experiment 6.1 suggest that cultivars can also be screened for storage potential and soaking injury on the basis of damage inflicted on germinability due to soaking in distilled water.

7.8.2. CULTIVARS OBTAINED FROM PAKISTAN

Eight cultivars obtained from the Agricultural Research Station at Mingora, Pakistan, differed in initial germination. High initial germination was reflected in resistance to accelerated ageing in most cultivars, but this was not absolute (experiment 6.2). The results were consistent with the findings of experiment 6.1 and findings of Kearns & Toole (1939). Moreover, Delouche (1982) arrived at similar conclusions. Cultivars Cumberland and Swat-84 (large seeds) were susceptible to accelerated ageing, but cultivar Epps and Pixie (large seeds) showed resistance to accelerated ageing compared to medium seeded cultivars 79-W-220 and 80-B-4007. The fact that cultivars with greater 100 seed weight were associated with longer shoot length and greater shoot fresh and dry weight has been previously established by Armstrong *et al.* (1988). To some extent results of experiment 6.2 and 6.3 agree to the findings of Armstrong *et al.* (1988). However, this relationship was not absolute.

Dassou & Kueneman (1984) subjected different soybean genotypes to incubator ageing or field weathering and observed that cultivars with large seeds were susceptible to accelerated ageing. However, in the current study some cultivars with large seeds were resistant to accelerated ageing compared to cultivars with medium sized seeds. The reason for this was probably the waxy or smooth hard seed coat in cultivars with large seeds. However, this was only true in case of the cultivar Epps, because better storability of the large seeded cultivar Pixie over medium seeded cultivars could not be attributed to differences in seed coat. Cultivar Weber (small seeds) had a smooth seed coat and showed resistance to accelerated ageing (Dassou & Kueneman, 1984).

As mentioned earlier, the cultivars studied in experiment 6.2 were obtained from Pakistan. Weber is a small seeded, short duration cultivar and has been reported to be suitable for spring planting in the plains of Pakistan, whereas, Epps is a medium seeded long duration cultivar and has shown its suitability for summer planting (PARC, 1990).

Holy & Gamble (1987) reported better field performance in small seeded soybean. Similarly after accelerated ageing (experiment 6.2) the small seeded cultivar Weber produced the maximum number of normal seedlings followed by the cultivar Epps, but for shoot fresh weight and shoot dry weight Epps and some other less storable cultivars exceeded Weber. However, for comparative loss in shoot fresh weight and shoot dry weight the cultivar Weber suffered least compared to other cultivars. Therefore, cultivars possessing seed characteristics similar to these two cultivars will probably perform better under tropical conditions. Small seed size has been reported to be an effective criterion for superior field establishment in pearl millet varieties (Lawan *et al.*, 1985). However, from the results of experiment 6.2 it is clear that seed size is not an absolute criterion of germination speed and improved seedling establishment in soybean.

Howle & Caviness (1988) reported that soybean seeds produced from the upper half of the plant exerted a greater emergence force compared to those collected from the lower half of the plant. They further reported that seedlings obtained from pods with only 2 seeds vertically displace greater weight than seedlings produced by seeds from pods with 3 or 4 seeds.

The results of experiment 6.2 were consistent with those of experiment 6.1, in suggesting that besides smaller seed size, smooth and waxy seed coats are a useful criterion for selecting cultivars for better storability. Hill, *et al.* (1986b) reported that differences in seed coat structure could be attributed to genetic differences and moisture availability at seed filling stage. This means that beside plant breeding,

modifications in production technology during the growing season can be used as an alternative approach to induce the character of hardseededness into soybean cultivars. This indicates that the character of hardseededness is not entirely under genetic control. Preharvest environmental conditions play an important role (literature review). In other words, if exposed to moisture stress at seed filling stage cultivar A may overcome the genetic superiority of cultivar B as far as the character of hardseededness is concerned. As legume seeds lose moisture there is a tendency for the testa to become impermeable to moisture ingress. Ellis *et al.* (1988) described this phenomenon as hardseededness.

Hardseededness may be reversible or irreversible. In the reversible case, the seeds initially fail to imbibe, but after a delay of several hours or perhaps several days the seeds start imbibing water and germinate. However, in case of irreversible hardseededness (undesirable character) the seeds fail to imbibe for considerable time when placed in a germination medium. The impermeability of the seed coat or testa stops the seed from germination. Cultivar Pb-1 studied in experiment 6.1 or Weber and Epps in experiment 6.2 had a slight (1-2%) tendency towards hardseededness and consequently showed comparative resistance to ageing.

As a result of the evidence mentioned in the literature review and findings from experiments 6.1 and 6.2, certain seed coat characteristics such as hardseededness, yellow seed colour, smoothness of seed coat, smaller size of the seeds, and smooth waxy type seed coats are associated with good storage potential. These findings will help in the selection of cultivars for storage potential on the basis of seed characteristics.

7.8.3. CULTIVARS OBTAINED FROM USA

In case of the experiment performed on nine cultivars obtained from Maryland, USA, cultivar Kent initially produced 10% fewer numbers of normal seedlings compared to

others (90%). In addition high initial germination before ageing did not indicate high storability in some cultivars. There was no clear differentiation between different seed size groups as far as storage potential, shoot fresh weight and solute leakage is concerned (experiment 6.3). Therefore, storage potential may not be entirely governed by seed size. In most soybean varieties the seed coat regulates water uptake in the initial hours of imbibition, but eventually serves as a reservoir of water for the hydrating seed (McDonald *et al.*, 1988b). However, in the initial hours of imbibition cultivars with small seeds that are normally associated with hard seed coats showed the impermeable response more strongly compared to large seeded cultivar that are normally associated with soft seed coats. Similar results have been reported by other workers mentioned in the literature review in chapter 1.

After investigation of the American cultivars it is again suggested that compared to seed size the characteristics of the seed coat may play a more vital role in controlling solute leakage and storage potential. These results agree with the findings of Kuo (1989), who observed that soybean cultivars with soft seed coat were susceptible to soaking injury compared to those with had hard seed coats.

The large seeded cultivar Emerald that had a green seed coat and oblong seeds, and York with lighter yellowish seeds showed high initial germination, but experienced a drastic decline in the number of normal seedlings after accelerated ageing. Cultivar Ripley with small seeds was the most resistant to accelerated ageing others were moderately resistant. Resistance of small seeded cultivar Ripley to accelerated ageing could be due to its higher percentage of seed coat by weight. Calero *et al.* (1981) gave similar reasons for resistance in small seeded cultivars to accelerated ageing.

Cultivars most susceptible to accelerated ageing showed a rapid decline in shoot fresh weight that was associated with higher solute leakage. Except for the Emerald, large seed size was associated with higher shoot fresh weight, however, shoot fresh

weight in some cultivars with small seeds was higher than those with medium seeds.

Some of the important seed coat characteristics that are likely to affect (a) moisture exchange between seed and the environment (b) the rate of water uptake by the seed and, (c) the impacts of mechanical abuse on the seed, are (1) seed coat thickness (2) the adherence of the seed coat to the embryo (3) the colour of the seed coat. Although, observations regarding the thickness of the seed coat were not made; it was observed that small seeded Ripley, in which the adherence of the seed coat was tighter to the embryo, showed resistance to accelerated ageing or soaking damage.

7.8.4. SEED VIABILITY AND SURVIVAL CURVES

The two major factors that affect the rate of seed deterioration are storage temperature and seed moisture content/relative humidity (Tekrony *et al.*, 1993). However, differences in longevity are often observed between genotypes produced and stored in the same environment. Similarly frequently, different seed lots of a cultivar may differ in seed vigour and viability even if produced in the same environment (Ellis, 1982). Ellis (1982) also reported that seeds produced from the same variety in different environments i.e. 2 or more geographical locations, may also exhibit great differences in vigour and viability. The environmental conditions prevailing during the formation and maturation of the seed can influence subsequent survival curves. Unfortunately very few studies have investigated the effects that factors like seed lots, cultivars, provenance (place of origin) and season can produce on seed vigour and viability (Mackay and Tonkin, 1967).

Seed deterioration curves are extremely useful in studying the storage behaviour of seed lots and predicting storage life (Nellist, 1981). In the current study (experiment 4.2) seeds sample at 8%, 12% and 16% moisture content were stored at 35°C or 40°C. The resulting seed deterioration curve is a function of storage

environment after 21 days (Appendix, Figure 4.1; Page 240). Conversely between cultivar differences in initial mean viability and viability after accelerated ageing were studied in 8 genotypes obtained from Pakistan and 9 genotypes obtained from Maryland USA (experiment 6.2 & 6.3). These cultivars lie on the viability curve (Appendix, Figures 6.2g & 6.3g; Page 240) in descending order according to their germinability to indicate if a cultivar that showed improved initial germination (due to seed size and seed coat colour etc.) retained its superior germinability after accelerated ageing.

The current research did not study if there are any differences in viability between seed lots of a cultivar produced in different environments or between seed lots of a cultivar subject to different storage environments. The effect of provenance on seed survival was also not studied. However, these aspects of seed viability in soybean are recommended for future research. Previous researchers have shown that environmental effects before, during and immediately after harvest and provenance produced a marked effect on potential viability periods of seed lots. For example Mackay and Tonkin (1967) found that the mean viability period of red clover (*Trifolium pratence* L.) derived from English sources was about 18% less than that derived from Canadian and New Zealand sources. They also found seasonal differences in the longevity of wheat, barley and oats harvested in England. It was observed that grain harvested in years of above average sunshine and below average rainfall lasted better than grain harvested in other less favourable years. The differences found in the mean viability period in lots derived from good and bad (weather) years were as much as 8% in oats, 14% in wheat and 24% in barley. Roberts (1973a) commented that the range of differences in viability for different seed lots reported by Mackay and Tonkin (1967), may be expected in practice in a number of common agricultural crops. Due to the complexity involved in studying these aspects few, if any experiments have been conducted to evaluate these

differences in soybean (Austin, 1972).

7.9. Conclusions

1. This study further confirmed that laboratory germination and tetrazolium staining test can be a good estimate of field emergence in seed lots that possess more than 80% normal seedlings under laboratory conditions, but may over-estimate field emergence of seed lots that exhibit less than 80% normal germination in the laboratory.

2. Sealed storage at low (6%) moisture content is ideal for monitoring germination. However, the seeds should be slowly hydrated to between 12 and 16% moisture content to avoid imbibition damage. Etched seed coats exacerbate solute leakage and reduce germinability.

3. Rapid water uptake decreased germinability and delays emergence. Reducing the rate of imbibition improved germination. Seeds imbibed in an inert osmoticum showed enhanced and improved actual germination. Mild moisture stress may not affect germination percentage, but early seedling growth is negatively affected.

4. Soybean seed size has been frequently reported to have a negative association with longevity in storage (Paschal & Ellis, 1978; Mugnisjah *et al.*, 1987). However, the current results have shown that seed size alone does not seem to be a reliable criterion for breeding purposes because not all small seeded genotypes provide improved storability.

7.10. Recommendations

This study led to the following recommendations that will be useful under field conditions in Pakistan.

1. Farmers in Pakistan do not know the vigour and viability level of their planting

seed and that has acted as a disincentive to the growing of soybeans. Results of experiment 1, led to simple test procedures that will provide an idea about the field planting value for a range of seed samples. However, researchers in Pakistan can use these tests as a base and confirm their accuracy for emergence under a range of field conditions. This will raise the confidence of farmers in their seed and motivate more and more farmers to cultivate soybean once uncertainty in emergence is replaced by confidence in acceptable crop stands.

2. Loss of vigour and viability in storage is a problem, but farmers in Pakistan do not understand about the moisture content of their seed for planting. However, these results have shown that farmers can slow down the rate of seed deterioration if they store their planting seed, sealed at 8% moisture content. On the other hand very dry seeds need to be raised to 16% or at least 12% moisture content before planting to avoid imbibition damage and obtain enhanced germination.

3. Cracks in the cotyledons should be avoided to minimise seed deterioration and solute leakage which otherwise reduces viability and vigour of seeds.

4. Seeds suffered high solute leakage or anoxia if kept submerged under water or polyethylene glycol (PEG). It is concluded that efforts should be made to avoid water uptake injury and prolonged saturated soil conditions. Rapid water uptake not only caused severe imbibition injury, but negatively affected growth rate during early seedling development, particularly in the case of aged seeds. Weaker seedlings may fail to emerge and if they do emerge, will result in a poor crop stand.

5. Seeds pre-imbibed in 25% PEG for 24 h enhanced germination of both unaged and aged seeds and increased the germination percentage in aged seeds. Pre-imbibing of seeds may have a role to play in improving germination and emergence in the tropics and subtropics.

6. Osmotic stress adversely affected germination in both aged and unaged seeds.

Seedling fresh weight decreased as osmotic stress increased. Efforts should be made not to expose germinating seeds to osmotic stress.

7. Cultivars that had a low rate of moisture absorption in a high relative humidity environment or resistance to water uptake injury exhibited better storage potential. The rate of hydration and/or resistance to soaking injury can therefore be used as a selection criterion in screening cultivars for good storage potential in tropical and subtropical countries. Together with small seed size; hard and yellow seed coats and less leakage can also be useful criteria for selection of cultivars for good storage potential. These simple procedures can easily be practised on farmers level.

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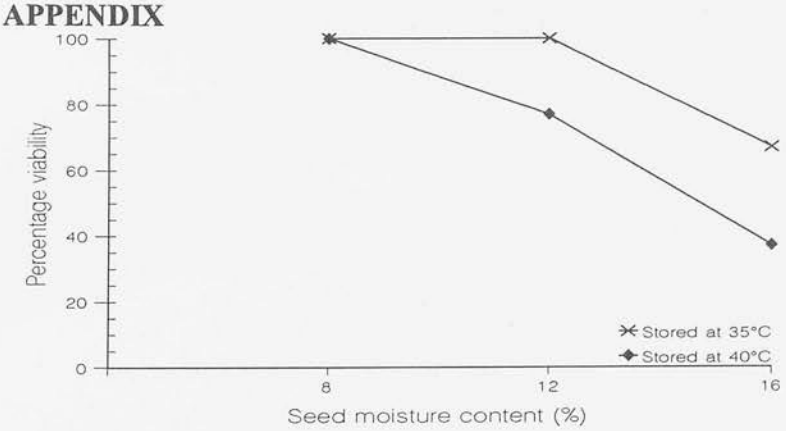

Figure 4.1. Seeds of cultivar Morgan after 21 days storage.

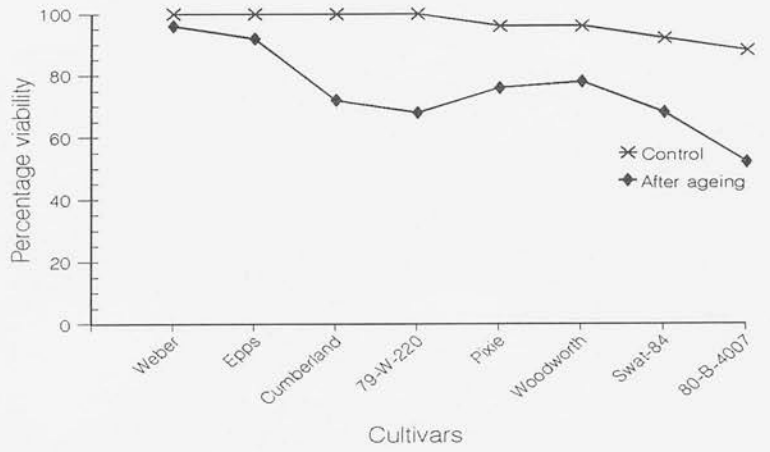

Figure 6.2g. Seed obtained from ARS, Mingora Pakistan.

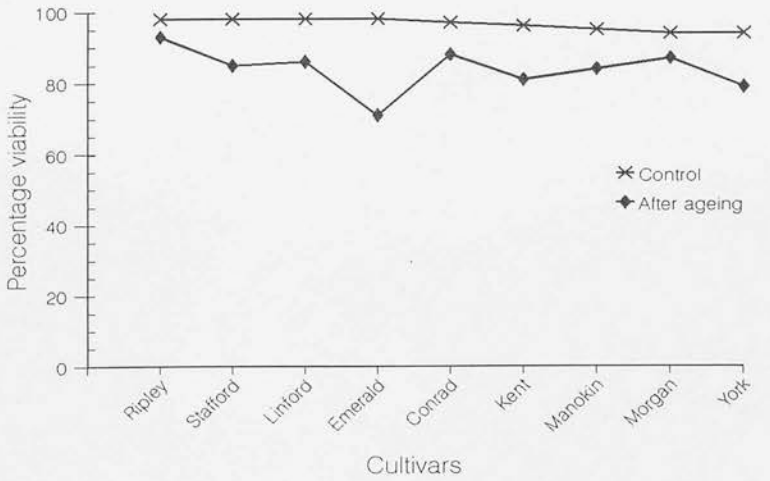

Figure 6.3g. Seed obtained from Maryland, USA.